Alternative Non-Linear Models for Fitting ELISA Curves

Todd L. Watterson & Chris Lyman
Quansys Biosciences
Logan, Utah

Introduction

Recent developments in personal computing and software have enabled scientists to utilize powerful techniques in nonlinear curve fitting and analysis. These advances extend to the use of non-linear curve fitting models in enzyme-linked immunosorbent assays (ELISAs). When fitting standard curves for ELISAs, many researchers opt for logistic models where the data on the x-axis is transformed, giving the curve a sigmoidal shape indicative of allostery. With chemiluminescent ELISAs, the signal amplification from the peroxidase reaction can result in greater sensitivity and the ability to detect and quantify lower antigen concentrations. Employed in multiplex fashion, the chemiluminescent ELISA is useful in measuring slight changes in cytokine levels and establishing baseline cytokine measurements in “normal” individuals. In order to maintain a wider dynamic range, the standard curve often employs greater dilution factors—or additional points are added—resulting in standard curves that have longer tails and asymmetric curves. The more common logistic models do not fit these curves well in the tail portion, often negating the increased sensitivity of the chemiluminescent ELISAs. Using curve-fitting software, alternative models—including peak models—were found that can accommodate the longer tails of such curves, allowing for enhanced detection of low levels of cytokines.

Materials and Methods

Cytokines were tested on 16-plex multiplex ELISA kits through Quansys Biosciences’ sample testing service. 1100 normal (presumed to be free of disease state) human samples were tested. Because the cytokine levels in the samples were believed to be low, a modification of the standard protocol was performed. This modified protocol included prolonged (2x) sample incubation times and altered standard curves. Antigen cocktail was initially diluted 1:16, and 12-point (including blank) standard curves were run. Exemplar curves were selected for additional analysis. Curve fitting was performed using multiple models and software packages, including the 5 parameter logistic equation and point-to-point or piecewise linear regression with Q-View™ Software (Quansys, Logan, UT). Alternative models were tested using SigmaPlot (Systat, San Jose, CA) or Prism 4 (Graphpad, LaJolla, CA). Optimal curves were determined by examining which curves gave the lowest value for PRESS statistic [1] or Akaike’s information criterion (AIC) value [2]. Lower limits of quantification (LLOQ) were determined as the lowest point of the standard curve whose back-fit value was within 20% of the actual value [3].

Results

The dilutions of the standards led to curves that measured at or near the detection limits of the assays (where there were no measureable increases in signal over that of the blank). Using the high sensitivity protocol alteration led to deterioration in the ability to quantify cytokine IL-17 at lower concentrations where the lower limit of quantification was 8.23 pg/ml with the modified protocol and was 2.74 pg/ml with the standard protocol. With IL-6, IL-10, and TNF-ß there was no change in sensitivity. With the remaining 12 cytokines, the altered high sensitivity protocol resulted in marked improvements in the sensitivity of the assays and the ability to quantify the cytokines at lower concentrations as evidenced by changes in the LLOQ values (Table 1). The 12-point dilution curves resulted in curves with longer tails (Figure 1) that were not all fit best by logistic curves. While nine of the curves were best fit by 5 parameter logistic (5PL) equation, others were best fit by peak model equations such as the Lorentzian 4 Parameter and Gaussian 4 parameter. It is interesting to note that IL-6 was fit best by a one-site saturation model—a sandwich ELISA has multiple binding sites involved in the process—which might be indicative of measurement of the kinetics of one predominant binding event. Piecewise linear regression proved to be almost as effective at improving sensitivity as the onerous process of determining optimally fitting curves.

Table 1: Lower limits of Quantification comparing the standard versus a modified protocol

Current Protocol					Modified Protocol					
Cytokine	**High**	**Low (LLOQ)**	**Model**		**Cytokine**	**High**	**Low (LLOQ) 5**	**LLOQ Point to Point**	**LLOQ**	**Model**
IL-1α	6500	8.92	5 parameter logistic		IL-1α	722.222	0.991	0.991	0.991	5 Parameter logistic
IL-1β	15000	20.58	5 parameter logistic		IL-1β	1666.667	6.85	0.762	0.762	Lorentzian, 4 Parameter
IL-2	2700	3.7	5 parameter logistic		IL-2	300.000	0.412	1.235	0.412	5 Parameter logistic
IL-4	2000	2.74	5 parameter logistic		IL-4	222.222	0.914	0.102	0.914	Lorentzian, 4 Parameter
IL-5	1700	2.33	5 parameter logistic		IL-5	188.889	0.086	0.259	0.086	5 Parameter logistic
IL-6	2500	3.43	5 parameter logistic		IL-6	277.778	3.43	1.143	1.143	One Site Saturation
IL-8	1000	1.37	5 parameter logistic		IL-8	111.111	0.457	0.457	0.457	Lorentzian, 4 Parameter
IL-10	1200	1.65	5 parameter logistic		IL-10	133.333	1.65	1.65	0.254	5 Parameter logistic
IL-12p70	5000	6.86	5 parameter logistic		IL-12p70	555.556	0.254	0.254	0.254	5 Parameter logistic
IL-13	2500	3.43	5 parameter logistic		IL-13	277.778	0.381	0.138	0.127	Lorentzian, 4 Parameter
IL-15	4000	5.49	5 parameter logistic		IL-15	444.444	1.829	1.829	1.829	5 Parameter logistic
IL-17	2000	2.74	5 parameter logistic		IL-17	222.222	8.23	0.914	8.23	5 Parameter logistic
IL-23	80000	109.74	5 parameter logistic		IL-23	8888.889	36.58	36.58		Lorentzian, 3 Parameter
Interferon γ	600	0.82	5 parameter logistic		Interferon γ	66.667	0.274	0.274	0.274	5 Parameter logistic
TNF α	2300	3.16	5 parameter logistic		TNF α	255.556	1.052	3.155	1.052	5 Parameter logistic
TNF β	6000	8.23	5 parameter logistic		TNF β	666.667	8.23	0.914	2.74	Gaussian, 4 Parameter

Figure 1

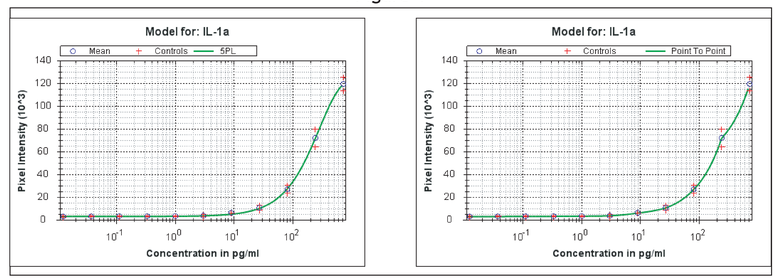
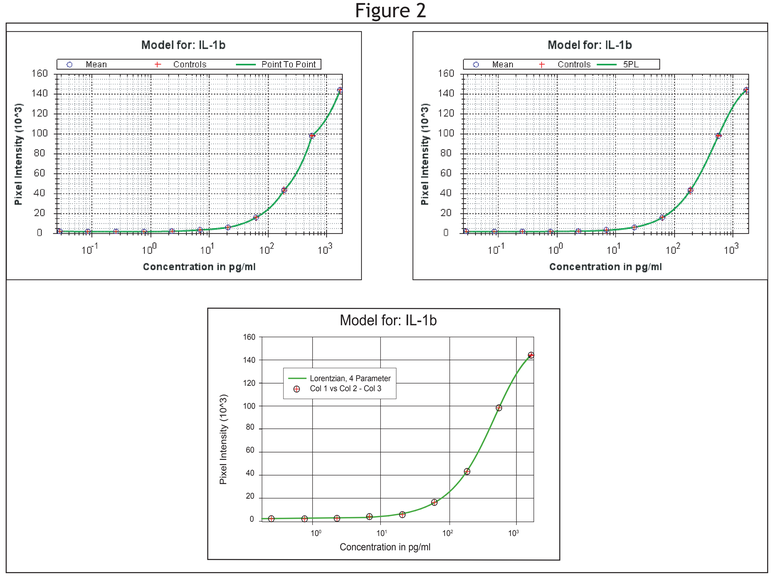


Figure 2



Conclusion

Modification of the standard Q-Plex™ array protocol has allowed for greater sensitivity and the detection of low levels of cytokines in sera from “normal” patients. Such modifications would be helpful in establishing baseline levels for cytokines in human serum and would be beneficial in helping establish cytokine profiles as diagnostic tools. In order to obtain the best results, additional curve-fitting tests proved to be helpful. The long-tailed curves were fit well by the 5 parameter logistic model in many cases. Where the logistic models were not the most appropriate, peak models worked well. In most instances, point-to-point or piecewise linear regression proved to be as effective as the best fit curves and, in some cases, proved to be better at quantifying low levels of cytokine.