《招待講演 4》

Improved Curve-fitting, Parallelism Testing, Characterization of Sensitivity and Specificity, Validation, and Optimization for Radioligand Assays

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A new computer program has been developed to simultaneously "fit" a family of curves, based on the four-parameter logistic equation. This provides an optimal test of "parallelism", permits estimation of shared parameters based on all of the available data, and provides "combined potency estimates". This method is especially useful for analysis of agonist-antagonist interaction at hormone and neurotransmitter receptors, DNA-RNA hybridization, and in-vitro bioassays.

Several simple general methods have been developed to permit testing of "parallelism" of standard and unknown preparations, irrespective of the method used for dose interpolation. For instance, one can utilize a weighted log-log least-squares linear regression between the amount detected per tube, and the volume of serum introduced into the test tube. One can then estimate standard errors for the "combined" potency estimates. These methods have been validated, and are readily adaptable to small desk-top computers.

The use of the mass-action law equations to describe RIA dose-response curves and for dose interpolation has been extended to apply to cases involving up to three independent classes of sites (one "non-saturable"), corresponding to a five-parameter Scatchard plot model. Parameters are estimated by weighted least-squares non-linear regression using a cubic equation relating bound-to-total ratio for ligand to the total ligand concentration. The program selects the "best" of the two-, three-, four- and five-parameter models; program "POTENT" is then used for dose interpolation. This method can readily be generalized to handle virtually any case of antibody heterogeneity, and positive or negative cooperativity, by use of spline fitting of the Scatchard plot or the binding isotherm.

The authors discuss a statistically meaningful definition of the minimal detectable dose in an assay. The detection limit is calculated by use of a one-sided Student's t-test in terms of the response variable with the appropriate number of degrees of freedom. This analysis should include the uncertainty in the response for zero dose as well as the uncertainty in the response for the "unknown".

Simple computer programs have been developed for analysis of the typical "recovery" experiment, i.e. when graded doses of unlabelled ligand are added to serum, to test the validity of the assay. We use a weighted regression, and test whether the intercept (a) is significantly different from zero and/or whether the slope (b) is different from unity. If appropriate, the program then calculates the weighted regression line forced through the origin. Related programs are used to compare the results from two different assay methods, when both measurements are subject to error.