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Software notice

Calculation of antibody and antigen concentrations from ELISA data using a graphical method

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A graphical method for determining the concentration of either the antibody or the antigen from ELISA data is presented in the form of a GWBASIC program. In the program, ELISAEQ, optical densities (OD) obtained from a 96-well ELISA plate can be input either directly by interfacing a microplate reader to the computer or manually. The program uses standard sample data, and selects the semilogarithmic linear range. Over this range, a least-squares method is used to determine the concentrations of interest. In addition, a hyperbolic interpolation formula is derived over the entire range for estimating the antibody or antigen concentration of the unknown samples whose OD is beyond the linear range.

Description

The enzyme-linked immunosorbent assay (ELISA) is often preferred for quantifying Ag/ Ab concentrations and many computer programs have been developed for this purpose (Canellas et al., 1981; Slezak et al., 1983; Caulfield et al., 1984; Mixter et al., 1986; Chalquest, 1987). In the ELISA procedure, a series of dilutions of known standards are included on the plate to create a standard curve and to derive an equation which would serve to estimate the Ab / Ag concentration of unknown samples. In earlier attempts, linear regression was used to fit straight lines to data and to compute a coefficient of linear correlation. Other attempts first used transformations, as the double reciprocal method of LineweaverBurk and various logistical methods (such as the four-parameter model and the logit-log plot), followed with linear methods applied to the transformed (linearized) data.

Hyperbolic regression (Studnicka, 1987) is a least-squares curve fitting technique that fits a set of data to hyperbolic curve without converting the equation to a linear form. Hyperbolic regression was used for ELISA optimization (Studnicka, 1991) and showed that the signal can be amplified by a factor of 2.5 with no additional noise, and the assay's useful Ab / Ag concentration range can be expanded five-fold.

The program described here combines the two approaches taking advantage of the increased sensitivity of the regression method when in the linear range. The program is so constructed that samples can be put in an ELISA plate in singlet, duplicate, triplicate, etc. Further, it is also applicable for calculating the signal, capacity, and flatness of the curve, which are required parameters for ELISA optimization.

Hardware / software requirements

Systems: IBM PC or compatible, under MSDOS, GWBASIC is required.

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Availability

From the journal's ftp site, Jim.mscs.mu.edu, under the directory pub /Raghava. A complete manuscript, paper.tex, in TEX technical typesetting system is also available.

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