JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1988, p. 147–148
0095-1137/88/010147-02$02.00/0

Vol. 26, No. 1

Quantitation of Indirect Sandwich Enzyme-Linked Immunosorbent Assay Parameters

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Received 9 March 1987/Accepted 30 September 1987

The optimization of data from the indirect sandwich enzyme-linked immunosorbent assay has been commonly accomplished by linear regression analysis, even though the data are often essentially sigmoid. A new microcomputer software program (LISACRV) that uses a nonlinear regression statistical model to analyze the data from enzyme-linked immunosorbent assay titration experiments was developed.

The enzyme-linked immunosorbent assay (ELISA) for the quantification of antigen and antibody was developed by Engvall and Perlmann (3) and has been proven to be a valuable technique with high sensitivity in a wide variety of serological assays (4, 8). ELISA is commonly used to assay the antibody level of a single serum sample but is also an important test for the differentiation of serologically defined virus strains and the measurement of antigen in a series of samples. The calculation of fitted titers from the optical density data with a logistical model has been reported to improve the accuracy of the test (6, 9). In both of these studies, the calculation of fitted titers was shown to correct the experimental fluctuations of individual tests and to improve the accuracy of the titers. The use of a logistical model appears to be a desirable procedure for improving the accuracy of an ELISA used for titrations.

The data derived from ELISA experiments have been analyzed by several different statistical models, with no single method clearly satisfying all the requirements of an ideal report (5). Hingley and Ouldridge (6) state that the most common type of logistical model used for the analysis of ELISA data is a linear type of model, and this has been confirmed by other workers (1, 7, 9). However, Hingley and Ouldridge (6) point out that in many cases, the data from ELISA are essentially sigmoid rather than linear, so a sigmoid type of analysis should be conducted. They developed a logistical model for a sigmoid type of analysis of such data.

The linear models that are commonly used (1, 7) as well as the sigmoid model that has been developed (2, 6) require the use of a large mainframe computer and sophisticated software, making them difficult for average laboratory clinicians to use. A new computer software program (LISACRV) that is based upon the logistical model of Hingley and Ouldridge (6) was developed to conduct a sigmoid analysis of data from the ELISA in the laboratory with a microcomputer.

LISACRV is compatible with any IBM-PC (International Business Machines Corporation) type of computer with a memory of over 65K. The calculations are completed in a few seconds and can be viewed on the screen or printed on an attached printer. An automatic portion of the program can be selected so that an unattended computer will complete the analysis and automatically print the results on an attached printer, normally on one sheet of paper. Each set of data produced by the program contains date and time markers to aid the orderly review of several experiments. A free copy of this compiled software program can be obtained from me.

The logistical model developed by Hingley and Ouldridge (6) had the following three parameters. (i) The average association constant is the reciprocal of the amount of substrate at the half saturation point and is an indication of the strength of binding between the individual reactive sites. (ii) The plateau value is the maximum optical density plateau value of the saturation curve and is a measure of the number of reactive sites on the material at fixed concentrations. (iii) The heterogeneity index is the value of the slope of the curve and is a measure of the heterogeneity of the reaction between the antigen and the antibody.

Computer inputs required. The operator of this computer program is only required to input six different entries: (i) the name or number of the experiment, (ii) the maximum number of iterations or halvings desired, (iii) the minimum residual increment desired, (iv) the total number of dilutions to be evaluated, (v) the log concentration of each dilution, and (vi) the observed optical density value for each dilution. The number of new items to enter is normally only four, since the maximum number of iterations can be entered as 200 and the minimum residual increment can be entered as 1.0. Both of these values are requested by the program to permit flexibility in its operation if desired. The operator can elect to enter the exact minimum residual increment as the value equal to 0.0001 times the squared maximum optical density value. This value is used in the program to determine the point for beginning another iteration of calculations.

Computer program output. The original observed optical density values that were entered are shown in Table 1. The observed optical density values used in Table 1 were identical to the data reported by Hingley and Ouldridge (6), who verified the accuracy of the original logistical model with 50 simulated experiments and 5 actual experiments with foot-and-mouth disease virus of cattle by using a fixed concen-

TABLE 1. ELISA saturation curve values, observed values, fitted values, and residuals[a]

Log concn	Observed optical density values	Fitted optical density values	Residuals
0.6	21	17.9021	3.0979
0.9	26	27.1863	−1.1863
1.2	35	38.66284	−3.662842
1.5	53	50.981	2.019001
1.8	64	62.35135	1.648647
2.1	71	71.47568	−0.4756775
2.4	78	78.0097	−9.69696E-03
2.7	82	82.31681	−0.3168106

[a] Serial correlation of residuals, −0.1588663; residual sum of squares, 31.54184.

TABLE 2. ELISA parameters[a]

Matrix of parameters	Avg association constant	Plateau value	Heterogeneity index
Covariance	2.698295E-05	−1.732982E-02	2.914783E-04
	−1.732982E-02	13.27019	−0.2319562
	2.914783E-04	−0.2319562	5.602166E-03
Correlation	1	−0.9158211	0.7496932
	−0.9158211	1	−0.8507254
	0.7496932	−0.8507254	1

[a] The final estimates of the calculated parameters were as follows: average association constant, 4.564094E-02; plateau value, 88.88547; and heterogeneity index, 0.807745. The standard errors of the fitted parameters were as follows: average association constant, 5.194512E-03; plateau value, 3.642828; and heterogeneity index, 7.484762E-02.

tration of antigen in the ELISA. The identical results obtained by LISACRV with these laboratory data verify its accuracy. The calculated fitted values of each of the original optical densities are also shown in Table 1, as are the appropriate statistical data that relate to the fitted optical density values. This statistical analysis was included to permit a review of the lack of fit of the model and to aid in the comparison of data from various experiments.

Table 2 contains the final estimates of the three parameters calculated by the software program: the average association constant, the plateau value, and the heterogeneity index. These parameters can aid in the understanding of the antigen-antibody reaction. The appropriate statistical analysis of these parameters was included to aid in the comparison of data from different experiments.

The computer program LISACRV provides a simple rapid technique for calculating fitted parameters of ELISA data and permits the analysis to be completed in the laboratory with a microcomputer by an operator with only a limited knowledge of computers. This program can aid in the standardization of the results of individual ELISA experiments and thereby improve the accuracy of the results. The application of LISACRV as a nonlinear logistical model should also be useful when the ELISA is used for virus strain differentiation, direct antibody assay, and titration of antigen when there is some evidence of saturation.

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