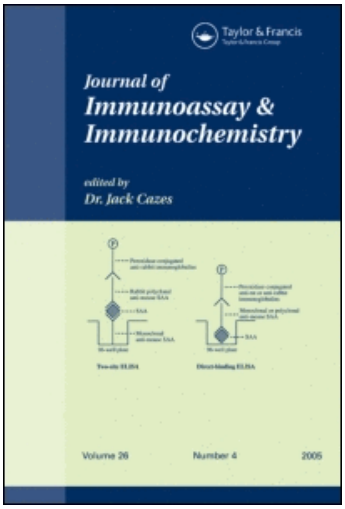

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Radioimmunoassay Data Processing with a Small Programmable Calculator

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RADIOIMMUNOASSAY DATA PROCESSING WITH A SMALL PROGRAMMABLE CALCULATOR

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ABSTRACT

We have developed three programs for data processing for radioimmunoassays RIA implemented on a small, inexpensive, programmable calculator (Texas Instrument Co., Model 59):

1) The first program performs a weighted logit-log regression for analysis of the RIA standard curve, and provides several descriptive statistics: slope, intercept, ED_{50} (and their corresponding standard errors), residual variance, and correlation coefficient. This program also provides: dose interpolation for unknowns; an estimate of the precision (percent coefficient of variation) for each result; corrections for variable sample volumes or recoveries; and the mean and standard error of the mean for samples analyzed in replicate.

2) The second program uses the "four parameter logistic model" to describe the dose response curve and perform dose interpolation. This is the equivalent to the use of the logit-log method, with provision for adjustment for the position of the 0% and 100% response. As such, it is more flexible and versatile than the logit-log method.

3) A third program is used for routine Within-Lab Quality Control: it calculates within-assay and between-assay precision, utilizing an analysis of variance (ANOVA) with a components of variance estimation. These programs incorporate the most important features of programs previously developed in this laboratory for an IBM 370 or a DECsystem-10 computer, and demonstrate the availability of adequate statistical analyses to laboratories without access to large centralized computer facilities.

Keywords: Radioimmunoassay, Immunoradiometric assay, Enzyme Multiplied Immunological Technique (EMIT), Enzyme Linked Immunosorbent Assay (ELISA), Quality Control, Logistic Model.

INTRODUCTION

Numerous methods have been developed for analysis of radioimmunoassay (RIA) results (1-3). Most of these programs were originally developed for large centralized computer facilities, e.g., IBM 370, DEC-10 and similar machines. Although such machines are available to large medical centers, they are not accessible to many RIA laboratories. Accordingly, over the past several years there has been considerable interest in adapting these programs to small desk-top calculators or minicomputers (1,4). Unfortunately, many of the adaptations to smaller machines have resulted in the use of inferior or even blatantly incorrect statistical methods and a severe loss of information. For instance, the logit-log method (5), which operates extremely well when proper weighting is employed, has been advocated either without weighting, or worse still, with inappropriate weighting. In the original logit-log method, the weights were described as a smooth continuous profile (Figure 1). In contrast, weights based on the observed sample standard deviations calculated from duplicates or triplicates leads to a very erratic weighting which is likely to be worse than use of an unweighted regression; it is almost equivalent to the use of random numbers for weights. Unfortunately several commercially available RIA programs have utilized this unstable and statistally unsound method.

The four parameter logistic model (6) offers several advantages and is more flexible than the original two-parameter logit-log method. This model is useful in analysis of data from bioassay, radioreceptor assay, *in vitro* bioassay, antibody dilution curves, and labeleled antibody assays as well as for RIA data. It may also be used for such diverse applications as estimation of molecular weight using gel filtration, or polyacrylamide gel electrophoresis in buffers containing sodium dodecyl sulfate (7). The analysis of quality control samples should provide estimates of within-assay, between-assay, and between-laboratory precision based on the principles of "analysis of variance" with a "components of variance" breakdown

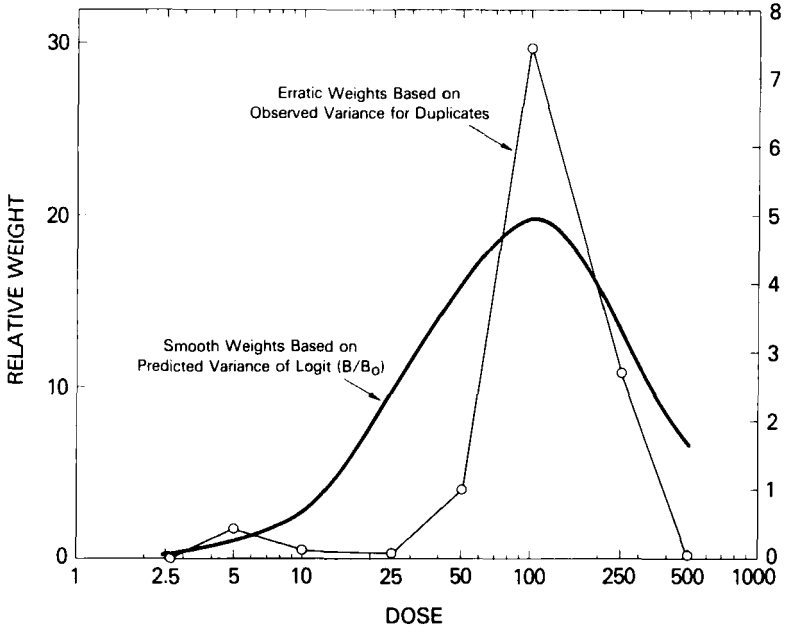

FIGURE 1. Weighting profile for logit-log regression. Ordinate: weights for each point; Abscissa: dose (log scale). Smooth curve: weights predicted on the basis of eq. 2, using J=1.5, generate a smooth weighting profile. Solid circles: weights based on the observed variance of logit (Y). This method introduces severe random noise and does not effectively use information from all of the dose levels: if two duplicates were, fortuitously, to agree perfectly, they would receive an "infinite weight" and processing would stop (division by zero).

(8-10). Unfortunately, the simple standard deviations can be misleading, unless one specifies whether the measurement of precision applies to a result based on single tube, duplicates, or on several replicate observations. Accordingly, we felt that it would be desirable to have reliable programs available on a small programmable calculator.

PROGRAMS

I. Logit-Log Method:

Here the sigmoidal log-dose response curve is transformed, yielding a linear relationship:

$$\text{logit}(Y) = \alpha + \beta \log X \qquad (1)$$

where:

$Y = B/B_o$ = normalized responce between 0 and 1

α = intercept

β = slope

$$\text{logit}(Y) = \log_e \left(\frac{Y}{1-Y} \right)$$

Although the original logit-log method utilized an iterative procedure with the weights and "working logits" adjusted after each iteration, we have found in practice that a single iteration for adjustment of weights is usually satisfactory. We have also found it possible to eliminate the initial unweighted regression, and simply use a single weighted regression, with weights calculated as a smooth function of the observed Y value:

$$w = \frac{[Y(1-Y)]^2}{\sigma_y^2} = \frac{[Y(1-Y)]^2}{a_o y^J} \qquad (2)$$

where y = raw counts, $Y = B/B_o$, and a_o, J are constants (11). The denominator corrects for non-uniformity of variance of the raw counts, while the numerator compensates for the effects of the logit transformation. A typical weighting profile is shown in Figure 1. The program provides the slope, intercept, ED_{50}, $\log(ED_{50})$, with their standard errors, and two measures of "goodness of fit", i.e., the residual variance (σ_o^2), and the correlation coefficient. The program provides an estimate of the expected percentage error for the potency estimate for each unknown, correction for variable sample volumes and/or recovery,

provision for use of either the Bound or Free counts, and the mean and standard error of the mean (SEM) for samples analyzed in replicate.

II. Four Parameter Logistic Method (6). This program performs essentially the same analysis as the logit-log program, with several important enhancements. In addition to adjusting the slope and ED_{50}, this program adjusts the "endpoints", i.e., high and low dose asymptotes of the dose-response curve:

$$y = \frac{a - d}{1 + (X/c)^b} + d \qquad (3)$$

where:

y is the dependent (response) variable (preferably raw counts).
X is the independent variable.
a,b,c,d are the four parameters:

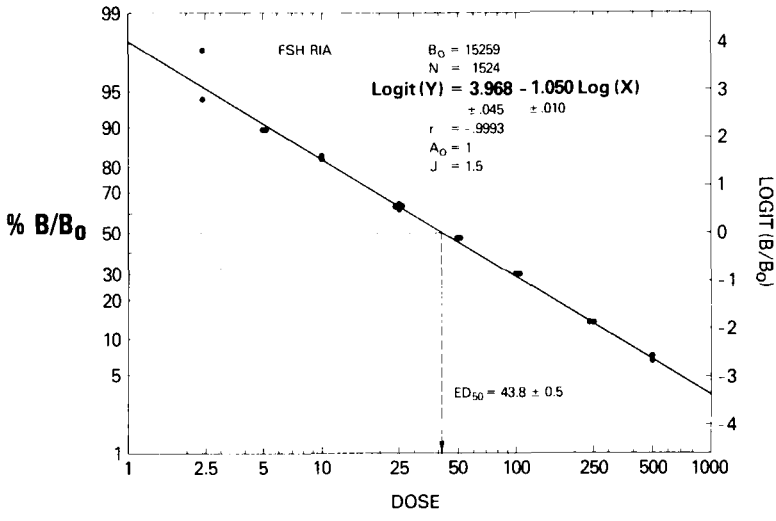

FIGURE 2. A. Calibration curve based on logit-log regression.

a Response for zero dose (corresponding to B_o)

b -1 × slope of the logit-log plot (slope factor which determines the steepness of the Dose-Response curve)

c ED_{50}, the value of X resulting in a response (Y) which is 1/2 way between a and d

d Response for infinite dose (often corresponds to nonspecific counts, N).

Many of the RIA standard curves which are not linearized satisfactorily by the logit-log method, can be adequately described by the logistic model. To adapt this curve-fitting method to a small calculator, we use an algorithm developed by one of us (PJM): Two successive linear regressions are used; first a logit-log regression to estimate b and c (slope and

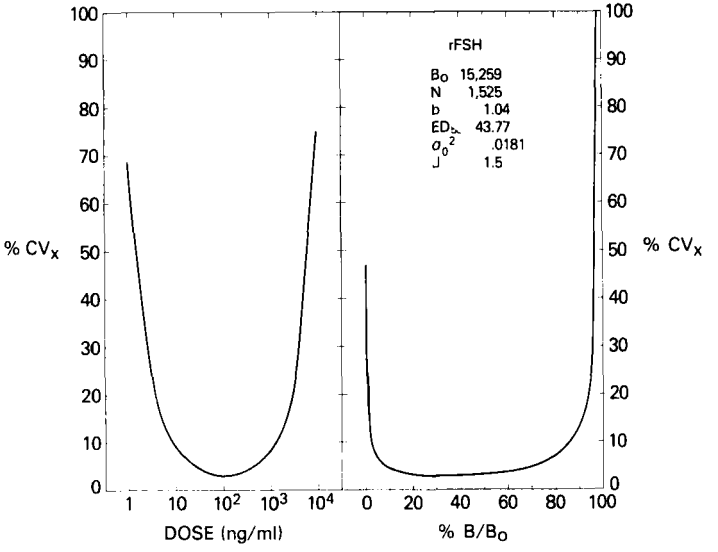

FIGURE 2. B. Plot of $\%CV_x$ for an unknown vs dose (X).
C. Plot of $\%CV_x$ for an unknown vs $Y=B/B_o$.

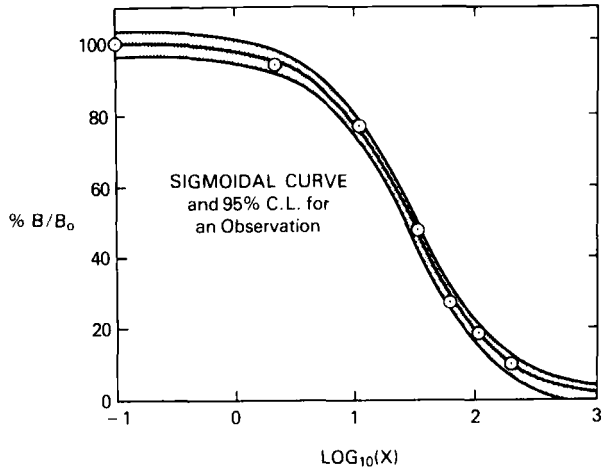

FIGURE 3. Calibration curve based on four parameter logistic program, showing ± 2 standard deviations for the response.

ED_{50}), and then a regression to estimate a and d (corresponding to B_o and NSB, in popular RIA terminology) (2). Since we fit two parameters at a time, and because of the simplicity of this approach, we call this the "2 • 2 Method". Smooth predicted weights are used for the logit-log regression,

$$w = [Y(1-Y)]^2 \qquad (4)$$

thus in effect assuming uniformity of variance for y (J=0 in eq. 2). The program provides estimates and standard errors for a, b, c and d in equation 3, and the root mean square (RMS) error of the fit. A second segment of this program provides the predicted dose level for every point on the standard curve, and also prints out coordinates of the "best fit" curve together with its 95% confidence limits ($\pm$ 2σ). A third segment of this program is used for "dose interpolation", providing potency estimates and the coefficient of variation (%CV) for unknowns.

III. Quality Control Program: This program calculates the within and between-assay precision (cf. Appendix 1 of 3). It

Table I. Example of Input and Output of Quality Control Program.

Listing of The Sample Input

```
Y1 Y2?
        74.00      Y1
        70.00      Y2
Y1 Y2?
        93.00      Y1
        93.00      Y2
Y1 Y2?
       105.00      Y1
       110.00      Y2
Y1 Y2?
       102.00      Y1
       102.00      Y2
Y1 Y2?
       105.00      Y1
        95.00      Y2
Y1 Y2?
        81.00      Y1
        81.00      Y2
Y1 Y2?
        78.00      Y1
        82.00      Y2
Y1 Y2?
        70.00      Y1
        70.00      Y2
Y1 Y2?
        87.00      Y1
        88.00      Y2
Y1 Y2?
        96.00      Y1
        83.00      Y2
Y1 Y2?
```

Listing of the Corresponding Output

```
Y1
        89.00      x̄        (average Y1)
        13.00      SD       (standard deviation)
         4.11      SEM      (standard error of the mean)
Y2
        87.40      x̄        (average Y2)
        12.95      SD       (standard deviation)
         4.09      SEM      (standard error of the mean)
YBAR
        88.25      x̄        (Grand mean)
        12.65      SD       (standard deviation of assay means)
         4.00      SEM      (standard error of the mean)
PRECISION -DUPS
         3.24      CVW      (Coefficient of Variation, Within-assay)
        14.33      CVB      (Coefficient of Variation, Between-assay)
INSTABILITY
        19.57      F        (Flag which appears when MS_b/MS_w is > 3)
COMPONENTS
         4.04      SW       (Standard deviation, Within-assay component)
        12.32      SB       (Standard deviation, Between-assay component)
```

provides the grand mean, within-assay coefficient of variation, and between-assay coefficient of variation. The program also tests for "parallelism": If the two replicates for each assay have been analyzed at two different dilutions, then a paired Student's *t* test is utilized to examine whether the mean value for the first replicate is equal to the mean for the second. If the ratio of the between-assay variance to the within-assay variance exceeds 3, a warning is printed, indicating that the assay system is unstable. Further, the within- and between-assay components of variance are provided, expressed as the standard deviation for a measurement based on a single tube. These values permit the user to predict the within- and between-assay variance for samples which have been analyzed in triplicate, quadruplicate, etc. The QC program can also be used to calculate within- and between-laboratory variability. Further, the calculations are applicable to any laboratory procedure performed in duplicate.

DISCUSSION

We believe that the present programs are superior in several respects to many previously available programs for desk-top and small calculators. By demonstrating the feasibility of incorporating a statistically valid weighting procedure using a small, inexpensive, portable programmable calculator, we hope to encourage the utilization of appropriate statistical methodology on equivalent or larger computing machines. We also demonstrate the feasibility of using the four parameter logistic method on a small programable calculator. The logistic method will fit most dose-response curves even when they are not adequately linearized by the logit-log method, and is also useful for labeled antibody assays, enzyme linked immunosorbent assays, bioassays, physiological dose response curves, DNA-RNA hybridization, and even for estimation of molecular weights by SDS-polyacrylamide gel electrophoresis and gel filtration (7,12).

One of the unfortunate consequences of the proliferation of smaller computing machines, has been the proliferation of

inferior quality or sometimes frankly incorrect software. As workers attempt to apply these methods to smaller machines, it is hoped that they will dispense with some relatively subtle refinements (e.g., iteration to adjust weights and working logits) but retain essential features (e.g., a smooth weighting profile based on predicted variance). It is tragic that the advent of widespread availibility of microprocessors, that many laboratories and equipment manufacturers have completely dispensed with any meaningful statistical analyses of the data, and have reverted to a primitive "point to point" or "connect the dots" approach to curve fitting (1). Indeed, computational methods were first introduced into the radioimmunoassay field in order to obtain a reliable, efficient method for statistical analysis (1-6). One can hope that the use of such primitive methods on microprocessors will be only a transitory phase. As the capability of these devices improves, and as the software improves, it should be possible to incorporate the most sophisticated analyses (12) into a small calculator , whether this be hand-held, desk-top, or an intrinsic part of the "RIA machine".

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Listings and details of the programs are available from:

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