Use of a Tabletop Computer for Antibiotic Assays

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The increasing need for rapid and accurate assay of antimicrobial agents in body fluids requires technical improvement of skills in these areas. A method for using a tabletop computer to simplify and shorten the statistical analysis of the laboratory data obtained by bioassay with the Olivetti Underwood Programma 101 has been developed so that a secretary or laboratory helper can rapidly develop the standard curves for each day's assays.

The use of a tabletop computer (Olivetti Underwood Programma 101) simplified the antibiotic bioassay method of Bennett et al. (1). When the diameter(s) of the inhibition zones of the standards are plotted against the log of the antibiotic concentrations, a parabolic curve develops. A polynomial regression is used to plot a best fit curve for the standards. Method 2 described by Bennett et al. can be simplified by using program no. 4.30 for a second degree trend (2) with the computer. The program uses the method of least squares to obtain the values for the constants of the polynomial and the required plotting coordinates.

The method of Bennett et al. (1) was used to assay antibiotics in human serum. The coordinates of the best fit line were calculated by using method no. 2 described by Bennett et al. and with the program. The results of a cephaloridine assay provide a typical example. The mean zone size for each standard and sample is calculated. The program card is inserted into the machine and the number of standards used is entered. In the example, five standards were used. The corresponding mean zone diameter for each standard is entered into the machine which automatically prints the three constants of the polynomial. The coded X value for the first standard, in this case, -2, is entered. The machine automatically prints a table of the plotting coordinates and continues the printout until cleared. The calculated zone diameters are plotted against the log, of the concentrations of the standards corresponding to the coded values used in the program.

For comparison, Table 1 shows the data for Bennett's method 2, and Table 2 depicts data obtained by the program. The calculated zone sizes are identical. A different coding system is used with the program, which yields different values for A and B in the polynomial. The machine automatically codes the standards for the calculation of the constants, but an understanding of the code is necessary to start the calculation of the plotting coordinates. The coding system is explained with the program (2), but in essence it is based on centering the X values so that the middle standard is set at X = 0. If an even number of standards is used, i.e., six, the mean of the two middle standards is set at X = 0. For six standards, -2.5 is entered instead of -2 to obtain the table of coordinates.

Unlike the equations published by Bennett et al., this program allows the use of six or seven standard solutions. This may be an advantage in performing assays on urine specimens containing antimicrobics with wide variations in their concentrations. The program will print values outside the data points of the standard curve, allowing dilutions for repeated assays to be extrapolated from the extended curve.

The use of this program has increased efficiency in the laboratory by decreasing the time needed to construct the standard curve. Laboratory helpers can operate the computer, since only seven numbers need be entered into the machine for the five standard assay design, thus freeing technical personnel for other tasks.
TABLE 1. *Results of method 2 of Bennett et al. (1) on a cephaloridine assay* *a*

| Actual concn (µg/ml) | Coded concn (X) | Mean zone diameter (Y) (mm) | Predicted zone diameter (Ŷ) (mm) |
|----------------------|-----------------|-------------------------------|----------------------------------|
| 0.5                  | 1               | 11.4                          | 11.6                             |
| 1.0                  | 2               | 15.9                          | 15.6                             |
| 2.0                  | 3               | 19.8                          | 19.8                             |
| 4.0                  | 4               | 24.0                          | 24.3                             |
| 8.0                  | 5               | 29.1                          | 29.0                             |

*Summation results: ΣY = 100.2, ΣXY = 344.1, ΣX²Y = 1364.7. Polynomial constants: A = 7.74, B = 3.71, C = 0.11. Regression equation: Ŷ = 7.74 + 3.71X + 0.11X².*

LITERATURE CITED

1. Bennett, J. V., J. L. Brodie, E. J. Benner, and W. M. Kirby. 1966. Simplified, accurate method for antibiotic assays of clinical specimens. Appl. Microbiol. 14:170-

TABLE 2. *Results of calculations by using the Olivetti program no. 4.30 on the same cephaloridine assay tabulated in Table 1* *a*

| Actual concn (µg/ml) | Coded* concn (X) | Mean zone diameter (Y) (mm) | Predicted zone diameter (Ŷ) (mm) |
|----------------------|-------------------|-------------------------------|----------------------------------|
| 0.5                  | -2                | 11.4                          | 11.6                             |
| 1.0                  | -1                | 15.9                          | 15.6                             |
| 2.0                  | 0                 | 19.8                          | 19.8                             |
| 4.0                  | 1                 | 24.0                          | 24.3                             |
| 8.0                  | 2                 | 29.1                          | 29.0                             |

*Polynomial constants: A = 19.82, B = 4.35, C = 0.11. Regression equation: Ŷ = 19.82 + 4.35X + 0.11X².*

*Coding is performed automatically.*

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2. Williams, J. B. Statistical analysis. Olivetti Underwood publication No. 6439.