DNA Concentrations in Serum and Plasma

To the Editor:

Davis and Davis (1) and Steinman (2) have reported finding no DNA in ethylenediaminetetraacetate (EDTA)-treated plasma, but large and variable amounts in serum. They suggested that serum DNA observed by other workers was released during clotting, and that only plasma DNA should be measured. However, using a highly sensitive and specific radioassay, we have found DNA as a normal constituent of both serum and plasma. Analysis of 66 normal human sera yielded low, rather heterogeneous, DNA values, with a mean of 318 ± 80 μg/liter (3). This material was nuclease sensitive, deoxyribonuclease I (EC 3.1.4.5) treatment of 11 normal sera yielded values not significantly different from the blanks (P > 0.10). Circulating DNA in corresponding amounts was also found as a normal serum constituent of all 10 animal species examined except the cat (4), which has a very high serum deoxyribonuclease activity (5). Comparison of DNA concentrations in serum and heparin-treated plasma from four individual Syrian hamsters indicated no significant differences. The individual values fell within 2 standard deviations of the method (CV, 8.9%), and the means were also not significantly different (serum, 124 μg/liter; heparin-treated plasma, 141 μg/liter; P > 0.10). Similar results were obtained for serum and heparin-treated plasma from three humans (respective means of 195 and 207 μg/liter; P > 0.10).

However, attempts to measure DNA in EDTA-treated plasma revealed a possible explanation for the reported (1, 2) absence of DNA from EDTA-treated plasma. Values for circulating DNA in such plasma samples were about a tenth those for the corresponding serum, with respective means of 35 and 388 μg of DNA per liter for 7 individuals (P < 0.002). This difference was partly due to inhibition by EDTA of the DNA extraction from the sample that is required before analysis. Extraction of 50 ng [3H]DNA from serum and heparin-treated plasma with phenol was essentially complete (hamster, 95% for both; man, 96 and 90% respectively), but was appreciably lower for EDTA-treated plasma (man, 77%). However, the decreased recovery of DNA during phenol extraction of EDTA-treated samples does not account for the 10-fold difference between paired samples of serum and EDTA-treated plasma, suggesting another mode of action. In any case, analysis of DNA was significantly inhibited by EDTA, the use of which should be avoided.

References


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Use of a Desk-Top Programmable Calculator in Method-Development Studies and Reference-Interval Determinations

To the Editor:

Calculations involved in method-development studies (1, 2) and in reference-interval determinations (3) are facilitated by computer processing. For method development, a graph of new-method values vs. reference-method values is necessary. For reference-interval calculations, a frequency histogram is desirable. This Letter describes a series of programs for a Model 600-14 programmable calculator (Wang Laboratories, Inc., Tewksbury, Mass. 01876) with peripheral Teletype, in which these graphs are a part of the output. Complete program listings, documentation, and instructions can be obtained upon request to me.

The program was written in four parts, each consisting of about 870 steps. Each part is loaded into memory from cassette tape as needed, beginning with Part 1. With this configuration, a maximum of 117 x,y pairs can be processed.

Part 1 consists of data entry and preliminary processing of the paired x,y values. For method comparisons, x is customarily the reference method value and y is the proposed new method value for each specimen. For reference-interval calculations, x is assumed to be age (or other parameter), and y is test value by the proposed method. The first section of output from Part 1 lists the following sequence of statistics: n, x, y, SD, (y - x)2, 2SD, x ± 1; 2SD, y ± 1; 2SD; (x1 - x2)2; 2SD; (y1 - y2)2; 2SD; (x, y); F-value; t-value for pairs; correlation coefficient; and slope, intercept, and standard error of the estimate for the linear regression of y on x.

The second section of Part 1 provides a list of the x,y pairs (sorted on y), their differences, and the y residuals of the linear regression line. For method comparisons, the column of differences facilitates the application of the sign test (2). The y residuals, together with a graph of the data pairs (see below) provide a basis for evaluation of goodness of fit and the identification of outlier points (1, 4).

Part 2 contains the program for printing a graph of the x,y pairs. Each axis contains 50 intervals. The number of points for any position is indicated by the digits 1 through 9. Ten or more overlapping points are indicated by an asterisk. The lower and upper limits for each axis can be selected from the keyboard, and only the data pairs within the selected limits will be printed. Two points for the linear regression line are indicated on the graph, so that this line can be drawn with a straight edge. The graph can be repeated with different limits for the axes as often as necessary, and without re-entry of the original data. The total number of points that are plotted on the graph and the coordinates for the linear regression line (which is always based upon the total number of points entered into the calculator) are printed below the x-axis. In addition to its obvious use in method comparisons, the x,y plot is useful for reference interval estimations. For example, a graph of age (x) vs. test value (y) might form the basis for splitting the reference interval into appropriate subdivisions by age.

Parts 3 and 4 are concerned with reference-interval calculations. The x, y pairs (age, etc.) of each x,y pair is erased during Part 3 to allow for additional