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Computer Simulation of Dose–Response Curves for Radioligand Assays and Related Procedures

To the Editor:
We consider the ability to perform computer simulations of dose–response curves for radioligand assays and related procedures as an important ancillary tool, useful both for promoting the understanding of the mathematical framework underlying assay design and for interpreting assay results. Accordingly, we have written a program for simulation of dose–response curves on a programmable desk-type electronic calculator (Olivetti Programma 101).

The program is based on a quadratic equation (1), derived assuming the applicability of the mass-action law, for the generally accepted ligand–receptor equilibrium interaction model for a univalent homogeneous antigen reacting with a homogeneous univalent antibody or binding protein (2). Our program solves the quadratic equation and gives seven response variables (B/F, F/B, B/T, B/Bo, B0/B, etc.) for a selected range of doses. We have used the program to evaluate the behavior of insulin radiomunnoassays and for the quantitative evaluation of insulin antibodies by radioimmunoassay. This program permits one to evaluate the effect on the standard curve of experimental manipulation such as: alteration of antibody concentration, alteration of labeled ligand concentration, or alteration of affinity constants (e.g., by change of temperature). The ability to perform these calculations quickly and easily with a small-capacity machine indicates that these analyses could and should be available to all radiomunnoassay laboratories.

We would be happy to provide the detailed description of the program, operating instructions, and sample input-output to interested persons.

References

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Ed. note: The information mentioned in the last sentence is also available from the editorial office of this journal, together with a 10-line BASIC language program, made available by D. Rodbard, that will be of interest to readers who now have available either commercial time-shared services or a desk-top computer such as the Wang 2200 or the Hewlett-Packard 9830, 31, or 45.

Mechanized Uric Acid Assay with an ABA 100 Analyzer According to Kageyama: An Evaluation

To the Editor:
The enzymic methods for assay of uric acid described by Kageyama (1) is commonly used. Deproteinization is not required and the method is based on measurement at 410 nm of the yellow color of 3,5-diacetyl-1,4-dihydropyrididine.

An adaptation of this technique with an ABA 100 automatic analyzer (Abbott Diagnostics, South Pasadena, CA 91030) is recommended to users of this apparatus.

Table 1 gives the results we obtained on assaying five sera with three different methods, using Boehringer reagent kits (Boehringer Mannheim S.A., Paris, France): the uricase ultraviolet method and the above-mentioned method used either manually or adapted to the ABA 100. Obviously, the results given by the automated process are always too high.

Rather than trying to assess the importance of this overestimate, which can vary from one serum to another, we have tried to determine its origin. Kageyama’s manual process involves use of a blank without uricase (EC 1.7.3.7) for each serum and a 60–70 min incubation. Under these conditions, the variations of absorbance recorded during incubation in the absence of uricase are compensated. In the mechanized method, in which sample blanks are not used, the differences of absorbance are measured between the 5th and 15th or 15th min of incubation. This methodology implies that the absorbance of the mixture serum and reagent without uricase is constant during the measuring period.

In fact, we have observed that the absorbance increases under these conditions, to an extent that varies from one serum to another and, expressed in uric acid concentrations, can be as high as 70 μmol/L. The blank values are particularly high with the control sera, Normal Validate (Precicio; Rueil-Malmaison France), but are negligible with the “Preciset” uric acid solutions, which explains the good linearity of the calibration curve.

Table 2 compares results of uricemia evaluations done by the manual method and its mechanized counterpart. Obviously, the differences between the results obtained with Kageyama’s original process and its adaptation to the ABA 100 are largely explained by the need to realize a true blank for each assay. Our observations confirm the results obtained by Haackel (2).

The mechanized method only takes into account a part of the absorbance variation as measured in the manual method, as shown by the kinetic study described previously (1, 2). With the aim of decreasing the relative magnitude of the blank, we have modified the procedure as follows.

Mode selector: Rate
Photometer displacement: PRR
Analysis time: 20 min
Carousel revolutions: 2

Under these conditions, a good calibration curve was obtained with Preciset. However, in the serum assays, these modifications did not improve the accuracy of the results, because the blank values increased concurrently. It therefore seems that adaptation of
Table 1. Urate in Five Sera, as Determined by the Uricase UV-method (A), Kageyama’s Manual Method (B), and Its Adaptation to the ABA 100 (C)

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<td>509, 529</td>
<td>519, 516</td>
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Each serum was assayed at least twice with each manual method.

Table 2. Urate in 10 Sera, as Measured by the Kageyama Method

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* A, manual method; B, ABA 100 method; C, ABA 100 method with the blank value subtracted (reagent without uricase).

Kageyama’s method to the ABA 100 analyzer may occasionally lead to errors as large as 70 μmol/L that we have observed without interference of hemolysis or bilirubin. However, it may be inferred that the methodology proposed by Haeckel (3), in which an aldehyde dehydrogenase is used, might be adapted satisfactorily to the ABA 100 (4).

References


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This communication was referred to Abbott Laboratories; a representative of that company offered the following comments.

To the Editor:

We have two abstracts from papers given at the Spring meeting of the Association of Clinical Scientists in Chapel Hill, NC, on May 2-4, 1975, and at the American Chemical Society, Pacific Conference on Chemistry and Spectroscopy in San Diego, CA, on November 1-3, 1973, by Dr. Paul Fu and Judy Duh (UCLA/Harbor General Hospital, Torrance, CA).

Both presentations relate that the precision studies, including day-to-day, showed a <2% coefficient of variation, and that statistical analysis of data from the ABA-100 applied to the Kageyama method as described in the Mizon Letter agreed well with the uricase ultraviolet 293-nm method as well as the SMA 1260 [continuous-flow] method (reduction of alkaline phosphotungstate).

According to Paz et al. (Med. Lab. Sci. 34, 163–166), the Kageyama method on the ABA-100 compared favorably with the manual carbonate–phosphotungstate method of Caraway, with a linear regression of y = 0.99x – 0.001 (y being ABA-100) and a correlation coefficient of 0.978. They also state that Kageyama reported interference by bilirubin and hemoglobin on the determination, requiring a blank for each sample to diminish these effects, but that separate sample blanks are not required with the ABA-100 kinetic approach.

We have also studied the method at Abbott Laboratories by repeating the experiment suggested in the Mizon paper and have found the following results. The maximum differences that we see between blank corrected and unblanked results are with quality-control serum; and the greatest difference, for four different control sera, was 35 μmol/L. For human serum, the greatest difference that we saw in 35 patients studied was 23 μmol/L and the overall mean differences between the two methods of blank and unblanked results give a difference of only 2.3 μmol/L. This would lead us to believe that if indeed there are differences between the two methods of unblanked and blank corrected results they are in fact very minimal and not considered significant when one considers the overall accuracy of automated methods for uric acid determination in the clinical laboratory.

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Improving Reliability of the Ratio LD-1/LD-2 in Diagnosis of Myocardial Infarction

To the Editor:

Determination of serum lactate dehydrogenase (LD; EC 1.1.1.27) isoenzymes and estimation of the ratio of LD isoenzyme-1 to LD isoenzyme-2 have been established as useful biochemical indexes in the diagnosis of myocardial infarction (MI), as further evidenced and supported recently by Leung and Henderson (7). They reported that the diagnostic specificity of LD isoenzymes for the detection of MI can be improved by standardization and increasing the reliability of the method used and by expressing the results as the ratio LD-1/LD-2.

Because changes of LD isoenzymes in serum are temporally related to the occurrence of MI it is important to consider the time intervals between the occurrence of MI and the collection of the serum samples for the proper interpretation of the results and their association with the clinical events. This information does not appear in the report by Leung and Henderson.

Method differences can account for variable results, as they point out. The diagnostic specificity of LD isoenzymes can be further improved by use of the same method to obtain normal patterns.