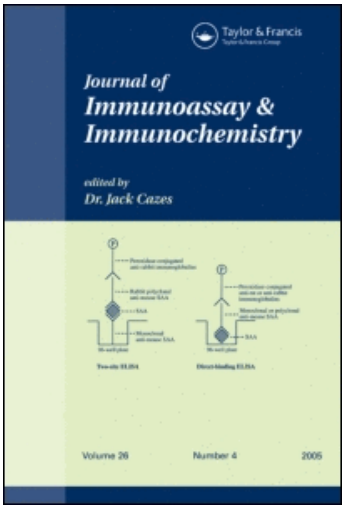


Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Data Processing for Radioimmunoassay Standard Curve Using Microcomputer: A New Basic Program for Weighted Logit-Log Transformation

Atsuo Murata[a]; Michio Ogawa[a]; Kazuhiko Matsuda[a]; Takeshi Kitahara[a]; Shunzo Nishibe[a]; Eiji Kurokawa[a]; Goro Kosaki[a]
[a] Second Department of Surgery, Osaka University Medical School, Osaka, Japan

To cite this Article Murata, Atsuo , Ogawa, Michio , Matsuda, Kazuhiko , Kitahara, Takeshi , Nishibe, Shunzo , Kurokawa, Eiji and Kosaki, Goro(1983) 'Data Processing for Radioimmunoassay Standard Curve Using Microcomputer: A New Basic Program for Weighted Logit-Log Transformation', Journal of Immunoassay and Immunochemistry, 4: 4, 407 — 423
To link to this Article: DOI: 10.1080/15321818308057018
URL: http://dx.doi.org/10.1080/15321818308057018

JOURNAL OF IMMUNOASSAY, 4(4), 407-423 (1983)

DATA PROCESSING FOR RADIOIMMUNOASSAY STANDARD CURVE USING MICROCOMPUTER: A NEW BASIC PROGRAM FOR WEIGHTED LOGIT-LOG TRANSFORMATION

Atsuo Murata, Michio Ogawa, Kazuhiko Matsuda, Takeshi Kitahara, Shunzo Nishibe, Eiji Kurokawa and Goro Kosaki

Second Department of Surgery, Osaka University Medical School, 1-1-50, Fukushima-ku, Osaka 553, Japan

ABSTRACT

A new BASIC program of the weighted linear, quadratic and cubic logit-log regression methods for data processing of radioimmunoassay standard curve was developed using a microcomputer. Using this program, three regression analyses are calculated and the table for root mean square of residuals in each regression are shown for the selection of the regression method. The data of three radioimmunoassays of human pancreatic enzymes and pancreatic secretory trypsin inhibitor were examined by this program and compared with those processed by the four parameter logistic method. The weighted cubic logit-log regression showed better fit in radioimmunoassays of human pancreatic enzymes with larger molecular weights. The present program is useful and practicable for its simplicity and accuracy.

(KEY WORDS: Radioimmunoassay, data processing, logit transformation, microcomputer)

INTRODUCTION

Since the development of the radioimmunoassay technique by Berson and Yalow (1), numbers of radioimmunological measurements

 0197-1522/83/0404-0407$3.50/0

for human peptide hormones, enzymes and special antigens have been increasing rapidly as routine works in clinical laboratories. In relation to the development, the data processing and the analytical methods for dose response curve of a newly developed radioimmunoassay have become one of the major problems in research works. Various programs have been available for data processing and curve fitting of the dose response curve of radioimmunoassay with several computer systems (2-6). However, most of these programs could be used in only limited laboratories because of the huge size and cost of computing machines, and thus the application of these methods is still restricted.

Recently with the high advances in the capabilities of small and inexpensive microcomputers, the intelligent and sophisticated analyses of the data processing have been accessible to many clinical laboratories and research laboratories (7).

The most popular and simple approach to linearization of the standard curve is the "logit-log" in which logit transformations of the response variables (B/B zero) are plotted against the logarithmic dose (8). In some cases of the newly developed radioimmunoassay systems, however, the logit transformation cannot lead to linearize the standard curve because of its unstable reactions of B zero, non-specific bound, and other fundamental problems. Therefore, the simple automated processing with the logit transformation might not be applicable to all the radioimmunoassay systems. Meanwhile, program-adjusted logit-log method (9), quadratic regression with the logit transformation (6), the

four parameter logistic function (2), the five parameter logistic function (10,11) and the empirical spline function (12) have been introduced instead of the linearization of the standard curve. However, these methods have been confirmed in only the radioimmunoassays of peptide hormones.

The aim of the present paper is to design a simple and easily-applicable program for radioimmunoassays of human enzymes using microcomputers. In order to obtain the appropriate curve in various types of radioimmunoassays, we used the weighted linear, quadratic and cubic regressions with the logit transformation. The program of these regression methods was written in BASIC language and partly written in the machine language for the bit image graphic subroutine.

MATERIALS AND METHODS

1. Radioimmunoassays

Radioimmunoassays of human pancreatic elastase 1 (13,14), urinary ribonuclease (RNase Us) (15) and pancreatic secretory trypsin inhibitor (PSTI) (16) were developed in our laboratory.

2. Equipment

The available equipments for the data processing are following machines; Sharp MZ-80C microcomputer having 48 kilobytes RAM with Epson MP-80II dot matrix graphic printer, and Sharp MZ-80K2E microcomputer also having 48 kilobytes RAM with Seikosha GP-80D

dot matrix graphic printer. Each of these microcomputers has a keyboard, 10 in. videomonitor and digital cassette recorder in one compact unit.

These machines accept programs written in BASIC language as well as in their own machine (Z-80 CPU) languages.

3. Mathematical and Statistical Methods

Each of assays was treated with the following logit transformation and the weighted regressions by the method of the least squares.

$$\text{logit } Y = A0 + A1 \log X$$

$$\text{logit } Y = A0 + A1 \log X + A2(\log X)^2$$

$$\text{logit } Y = A0 + A1 \log X + A2(\log X)^2 + A3(\log X)^3$$

logit Y is obtained for the relation;

$$\text{logit } Y = \log e\ (Y / 1-Y), \qquad Y = (B-NSB) / (B0-NSB)$$

where B represents count bound with given dose X; B0, zero dose count bound; NSB, count nonspecifically bound. The parameters A0, A1, A2 and A3 were calculated by the least square solution.

The weight adapted to these regressions was accompanied with the logit transformation and was the following equation;

$$Wi = Yi^2(1-Yi)^2$$

In order to test the "goodness of fit", the root mean square (RMS) for residuals was calculated with the following equation;

$$RMS = \sqrt{\frac{\Sigma(Yi-\bar{Y}i)^2}{N-P}}$$

Yi: observed $\frac{Bi-NSB}{B0-NSB}$

$\bar{Y}i$: predicted $\frac{Bci-NSB}{B0-NSB}$

where Bi represents bound count observed; Bci, bound count calculated by each regression; N, number of standard points; P, number of parameters.

4. Four Parameter Logistic Function

The four parameter logistic function introduced by Rodbard et al. (17) was also comparatively examined;

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

where y is response count; X, standard dose; a, the response when X=0; b, a slope factor; c, the dose which has a predicted response exactly half-way between the upper and lower plateau of the dose response curve; d, the predicted response for an infinite dose of unlabeled ligand, equal to nonspecific bound.

The BASIC program for this four parameter logistic model (NIH-RIA) was kindly supplied by Ms. Terri Shaw, Biomedical Computing Technology Information Center, Tenn., USA and was converted to our own BASIC language with slight modifications.

RESULTS

Fig. 1 shows the process chart for this program. Observed count data are entered manually from a keyboard and once filed

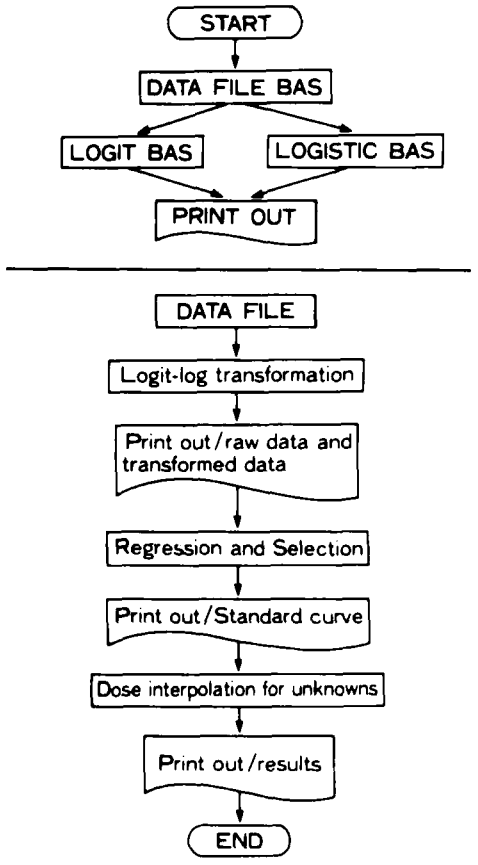


FIGURE 1. Process chart

with a proper file code into a cassette tape or a floppy disk (DATA FILE BAS). Then the filed data are processed to be calculated for B/B0 % and the logit transformation (LOGIT BAS). Three regression analyses for the logit-log model are calculated and the tables for RMS in each regression are shown to be selected.

Fig. 2 (a) shows a sample run for a radioimmunoassay of human pancreatic elastase 1. In this case, the cubic logit-log

model was the best regression curve. The four parameter logistic model is also shown in Fig. 2 (b). Two types of dose response curve are printed out in bit-imaged graphics.

Table 1 shows the root mean squares (RMS) for residuals in four types of regression models in six assays of human pancreatic elastase 1. The cubic logit-log model was the best regression model in these six assays. RMS in the four parameter logistic model revealed almost the same value as that in the linear logit-log model.

Table 2 shows the root mean squares in six assays of human RNase Us. The quadratic or cubic logit-log model shows better regression in this radioimmunoassay.

The root mean squares in six assays of human PSTI are shown in Table 3. In this assay, the linear logit-log or four parameter logistic model shows better regression.

DISCUSSION

The search and comparison of the different mathematical methods were reported by Vogt et al. (18), and Geier and Rohde (19,20) using radioimmunoassays of human peptide hormones, but no generalized approach of radioimmunoassays of human enzymes with larger molecular weight has been demonstrated. Therefore, in the development of radioimmunoassays of human enzymes, the linear logit-log method is used widely because of its simplicity and its theoretical background. However, in some systems of radioimmunoassays of enzymes, the logit-log method failed to provide adequate linearization. The antigen-antibody reaction in the radioimmuno-

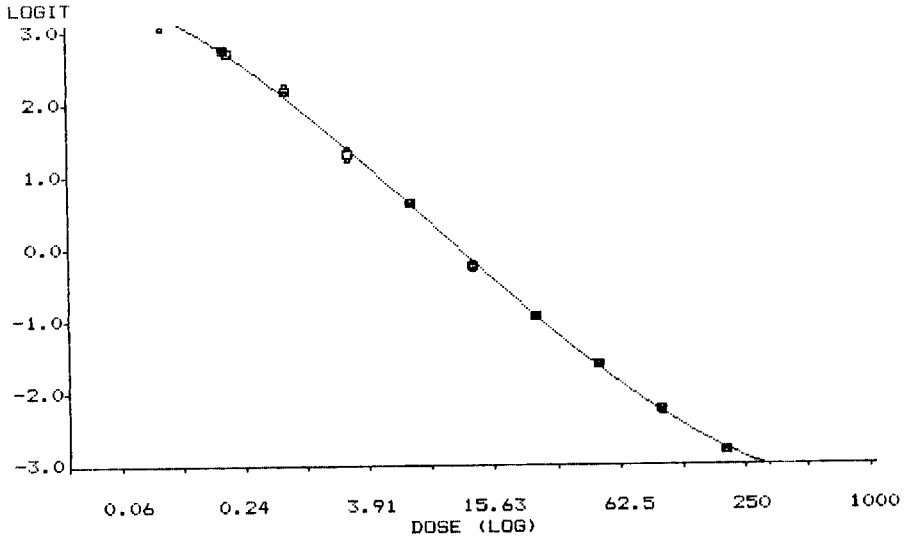
LOGITLOG

アナタ ハ 3 ジ ジキ ヲ シテイシマシタ。

$$LOGIT\ Y = A0 + A1*LOG(X) + A2*LOG(X)\uparrow 2 + A3*LOG(X)\uparrow 3$$

A0 = 2.5232174
A1 =-0.89234329
A2 =-0.1156739
A3 = 0.017920857

STANDARD CURVE



ED 20 = 37.83
ED 50 = 10.67
ED 90 = 1.42

FIGURE 2 (a). Sample run for a radioimmunoassay of human pancreatic elastase 1 processed by the logit-log model.

RESIDUAL TABLE

ID.	STD DOSE	STD COUNT	CALC B/BO	RESIDUAL
1 ｼﾞｭｼﾝ ﾃﾞｰﾀ ----				
1	200	915.5	0.05	0.01
2	100	1334.5	0.1	0
3	50	2096	0.18	-0.01
4	25	3346	0.3	-0.02
5	12.5	5077	0.47	-0.03
6	6.25	7404.5	0.64	0.01
7	3.125	8849	0.78	0
8	1.563	10077.5	0.88	0.02
9	0.781	10516.5	0.94	0
10	0.391	10747.5	0.97	-0.01
			<<< RMS =	0.016 >>>
2 ｼﾞｭｼﾝ ﾃﾞｰﾀ ----				
1	200	915.5	0.05	0.01
2	100	1334.5	0.1	0
3	50	2096	0.18	-0.01
4	25	3346	0.3	-0.02
5	12.5	5077	0.47	-0.03
6	6.25	7404.5	0.64	0.02
7	3.125	8849	0.78	0.01
8	1.563	10077.5	0.88	0.02
9	0.781	10516.5	0.94	0
10	0.391	10747.5	0.97	-0.01
			<<< RMS =	0.018 >>>
3 ｼﾞｭｼﾝ ﾃﾞｰﾀ ----				
1	200	915.5	0.06	0
2	100	1334.5	0.09	0
3	50	2096	0.16	0.01
4	25	3346	0.28	0
5	12.5	5077	0.46	-0.02
6	6.25	7404.5	0.65	0.01
7	3.125	8849	0.8	-0.01
8	1.563	10077.5	0.89	0.01
9	0.781	10516.5	0.94	0
10	0.391	10747.5	0.96	0
			<<< RMS =	0.01 >>>

Fig 2a (continued)

LOGISTIC

INITIAL VALUE FOR A = 11171.5

INITIAL VALUE FOR B = 1

INITIAL VALUE FOR C = 11.021379

INITIAL VALUE FOR D = 305

INITIAL RESIDUAL VARIANCE 40654.152

IT.	A	B	C	D	RES. VAR.
1	11107.782	1.106294	10.304287	499.89129	17122.352
2	11099.973	1.1225881	10.34356	498.17614	16470.245
FINAL	11099.973	1.1225881	10.34356	498.17614	16470.245
+/- 1 SE	75.588394	0.032464909	0.28854507	83.067659	
% ERROR	0.68097817	2.8919699	2.7896109	16.674355	

LOG10 'C' = 1.01467 +/- 0.012113555
UPPER & LOWER 95% C.L. FOR LOG10 'C' : 1.0401233 0.98921682
CORRESPONDING 95% C.L. ON 'C' : 10.967894 9.7547652

RMS ERROR = 128.33645

STANDARD CURVE

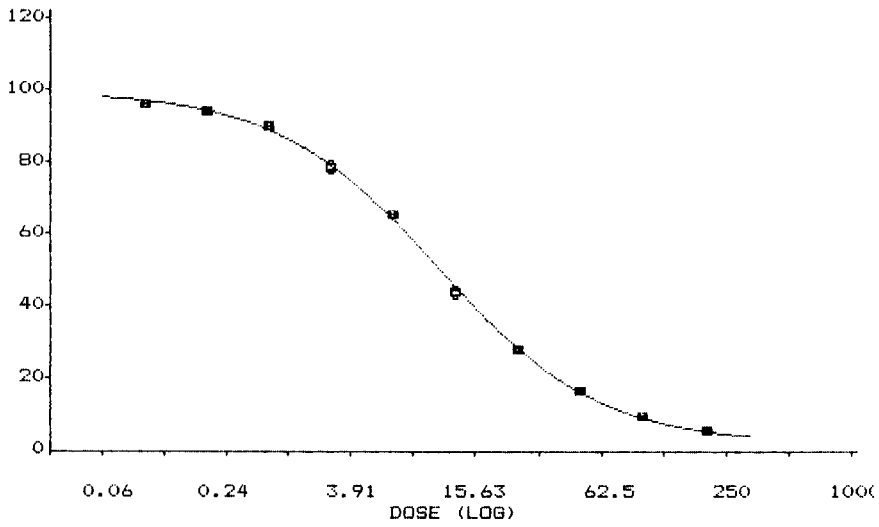


FIGURE 2 (b). Sample run for the same assay processed by the four parameter logistic model.

RESIDUAL TABLE

ID.	STD DOSE	STD COUNT	CALC B/BO	RESIDUAL
-1	9999	498.18	0	0
0	0	11099.97	1	0
1	200	866.29	0.034721641	0.02
2	100	1268.21	0.072632691	0.02
3	50	2042.75	0.14568965	0.02
4	25	3368.87	0.27077441	0.01
5	12.5	5237.76	0.44705464	-0.01
6	6.25	7259.3	0.6377335	0.02
7	3.125	8906.37	0.79309104	-0.01
8	1.563	9965.6	0.89300192	0.01
9	0.781	10547	0.94784133	-0.01
10	0.391	10838.64	0.9753499	-0.01

<<< RMS = 0.016 >>>

ED 20 =	38.353
ED 50 =	10.557
ED 90 =	1.4

Fig 2b (continued)

assay of human enzymes may be more complicated than that in the radioimmunoassay of peptide hormones. Therefore, the simple mass-action law (6,21) may not be adjusted to the radioimmunoassay of human enzymes and so the logit transformation may fail to linearize the dose response curve.

Furthermore, when the assay condition of a new radioimmunoassay system has not been settled, there are many problems in the tracer, the antibody, the cross-reaction and others (22). Therefore, a dose response curve is not always fitted with a single automated method for its regression.

The results obtained here indicate that, in the radioimmunoassay of smaller molecular weight protein (PSTI: 6,500), the linear logit-log model or four parameter logistic model shows the

TABLE 1

ROOT MEAN SQUARES FOR RESIDUALS IN FOUR TYPES OF REGRESSION METHOD IN RADIOIMMUNOASSAY OF HUMAN PANCREATIC ELASTASE 1.

	1	2	3	4	5	6
linear logit-log	0.0166	0.0323	0.0185	0.0326	0.0292	0.0139
quadratic logit-log	0.0174	0.0254	0.0137	0.0118*	0.0181	0.0141
cubic logit-log	0.0098*	0.0087*	0.0101*	0.0128	0.0170*	0.0089*
four parameter logistic	0.0161	0.0305	0.0148	0.0377	0.0288	0.0132

*: The least value of RMS in each assay

TABLE 2

ROOT MEAN SQUARES FOR RESIDUALS IN FOUR TYPES OF REGRESSION METHOD IN RADIOIMMUNOASSAY OF HUMAN URINARY RNase Us.

	1	2	3	4	5	6
linear logit-log	0.0322	0.0384	0.0316	0.0449	0.0383	0.0161
quadratic logit-log	0.0175	0.0176*	0.0072*	0.0161*	0.0291	0.0160
cubic logit-log	0.0154*	0.0280	0.0078	0.0161	0.0183*	0.0136*
four parameter logistic	0.0332	0.0396	0.0351	0.0434	0.0492	0.0162

*: The least value of RMS in each assay

TABLE 3

ROOT MEAN SQUARES FOR RESIDUALS IN FOUR TYPES OF REGRESSION METHOD IN RADIOIMMUNOASSAY OF HUMAN PANCREATIC SECRETORY TRYPSIN INHIBITOR (PSTI).

	1	2	3	4	5	6
linear logit-log	0.0221	0.0207	0.0142	0.0130*	0.0124*	0.0092*
quadratic logit-log	0.0198	0.0234	0.0152	0.0135	0.0124	0.0098
cubic logit-log	0.0228	0.0250	0.0152	0.0163	0.0125	0.0107
four parameter logistic	0.0161*	0.0200*	0.0138*	0.0134	0.0250	0.0183

*: The least value of RMS in each assay

most appropriate "fit". But, in the radioimmunoassays of larger molecular weight enzymes (pancreatic elastase 1: 30,000; RNase: 13,000), the logit transformation fails to linearize their dose response curves. From the view of mass-action law, the enzymes may have several sites of antigen-antibody reaction and various equilibrium constants for their reactions. This result could be supported by the examinations of Ichihara et al. (10,11) using radioimmunoassays of several peptide hormones.

The four parameter logistic method is the most theoretically-based model for radioimmunoassays, but, in the present study, RMS value of the four parameter logistic method shows larger in the assays of enzymes (pancreatic elastase 1 and RNase) than that of

the cubic logit-log method. The four parameter logistic method is mathematically the same as the linear logit-log method except the former method utilizes B0 and NSB as parameters. B0 and NSB in the developmental assay of enzyme may not be stabilized because of its complicated reaction. For example, serum immunoreactive pancreatic elastase 1 is not a free circulating pancreatic elastase 1 but alpha 1 - antitrypsin-bound pancreatic elastase 1 (13). Therefore, the antigen-antibody reaction in this radioimmunoassay may not be saturated or may be more complicated so that its dose response curve cannot be stabilized to only one regression method. In contrast, serum immunoreactive PSTI is a free circulating PSTI (16) so that its dose response curve is fitted well with the linear logit-log method or four parameter logistic method. Therefore, when the four parameter logistic method is used in a radioimmunoassay of enzymes, counts of B0 and NSB should be stabilized in replicates and the number of standard points should be done adequately.

The present program based on a simple assumption on the variance, uses an interpolating function with four parameters and simple mathematical computations. Thus, it can be concluded that this program is useful and practicable in many research laboratories for its time-saving, simplicity and accuracy.

SOFTWARES

Computer program written in BASIC with the method diagrams, listing and sample runs are available on request.

ACKNOWLEDGEMENT

We are grateful to Dr. David Rodbard, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, for his helpful advices on our manuscript, and to Ms. Terri Shaw, Biomedical Computing Technology Information Center, Nashville, Tenn., for sending us the program list of the four parameter logistic model (NIH-RIA) and many informations.

REFERENCES

1. Berson, S. A. and Yalow, R. S. Quantative aspects of the reaction between insulin and insulin-binding antibody. J. Clin. Invest. 1959; 38: 1996-2016.

2. Healy, M. J. R. Statistical analysis of radioimmunoassay data. Biochem. J. 1972; 130: 207-10.

3. Burger, H. G., Lee, V. W. K., Rennie, G. C. A generalized computer program for the treatment of data from competitive protein-binding assays including radioimmunoassays. J. Lab. Clin. Med. 1972; 80: 302-12.

4. Spona, J. Rapid assay for luteinizing hormone and evaluation of data by new computer program. In: Radioimmunoassay and Related Procedures in Medicine, 1974; 1: 123-30.

5. Grotjan, H. E. and Steinberger, E. Radioimmunoassay and bioassay data processing using a logistic curve fitting routine adapted to a desk top computer. Comput. Biol. Med. 1977; 7: 159-63.

6. Rodbard, D. Data processing for radioimmunoassay: An overview. In: Natelson, S., Pesce, A. J. and Dietz, A. A. eds. Clinical Immunochemistry, Chemical and Cellular Bases and Applications in Disease; Current Topics and Clinical Chemistry, Vol 3, Washington, D.C.: American Association for Clinical Chemistry, 1978: 477-94.

7. Davis, S. E., Munson, P. J., Jaffe, M. L., Rodbard, D. Radioimmunoassay data processing with a small programmable calculator. J. Immunoassay 1980; 1: 15-25.

8. Yalow, R. S. and Berson, S. A. General principles of radioimmunoassay. In: Hayes, R. L., Goswitz, F. A., and Murphy,

B. E. P. eds. Radioisotopes in Medicine, In Vitro Studies. Tenn. 1968: 7-41.

9. Hatch, K. F., Coles, E., Busey, H., and Goldman, S. C. End-point parameter adjustment on a small desk-top programmable calculator for logit-log analysis of radioimmunoassay data. Clin. Chem. 1976; 22: 1383-9.

10. Ichihara, K., Yamamoto, T., Kumahara, Y., et al. An improved processing of radioimmunoassay data by means of a desk top calculator. (1) Comparison of regression procedures applied to selected kinds of radioimmunoassay. Clin. Chim. Acta 1977; 79: 331-40.

11. Ichihara, K., Yamamoto, T., Miyai, K. An improved processing of radioimmunoassay data by means of a desk top calculator. (2) A study of indices for discrimination of standard curve with large aberrance. Clin. Chim. Acta 1977; 80: 37-47.

12. Vikelsoe, J. Spline-fits: An alternative route to computer treatment of data from competitive protein-binding assays. Acta Endocrinol. (Suppl) 1973; 147: 100.

13. Murata, A., Ogawa, M., Fujimoto, K., Kitahara, T., Matsuda, Y., Kosaki, G. Radioimmunoassay of human pancreatic elastase 1. Enzyme (in press).

14. Murata, A., Ogawa, M., Fujimoto, K., Kitahara, T., Kosaki, G. Changes in serum immunoreactive pancreatic elastase 1 in acute pancreatitis. Hepato-gastroenterology 1982; 29: 278-80.

15. Kurokawa, E., Ogawa, M., Kurihara, M., et al. Radioimmunoassays for two types of human urinary ribonucleases: Differential determination of ribonucleases in human serum. Clin. Chim. Acta 1983; 128: 83-93.

16. Kitahara, T., Takatsuka, Y., Fujimoto, K., Tanaka, S., Ogawa, M., Kosaki, G. Radioimmunoassay for human pancreatic secretory trypsin inhibitor: Measurement of serum pancreatic secretory trypsin inhibitor in normal subjects and subjects with pancreatic diseases. Clin. Chim. Acta 1980; 103: 135-43.

17. Rodbard, D. Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. Clin. Chem. 1974; 20: 1255-70.

18. Vogt, W., Sandel, P., Langfelder, Ch., Knedel, M. Performance of various mathematical methods for computer-aided processing of radioimmunoassay results. Clin. Chim. Acta 1978; 87: 101-11.

19. Geier, T. and Rohde, W. Comparison of four mathematical models for the calculation of radioimmunoassay data of LH, FSH and GH. Endokrinologie 1981; 78: 269-80.

20. Geier, T. and Rohde, W. A simple strategy convenient for processing of radioimmunoassay data and quality control in the laboratory on a desk-top-calculator. Endokrinologie 1981; 78: 281-96.

21. Rodbard, D. Mathematics and statistics of ligand assays: An illustrated guide. In: Langan, J. and Clapp, J. J. eds. Ligand Assay: Analysis of International Developments on Isotopic and Nonisotopic Immunoassay. New York: Masson 1981.

22. Walker, W. H. C. An approach to immunoassay. Clin. Chem. 1977; 23: 384-402.