

BRIEF NOTES AND COMMENTS

A Computer Program for Immunoassay of Protein Hormones, with Special Reference to Insulin and Growth Hormone

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SUMMARY

A Fortran language program suitable for use in computer processing of two-antibody radioimmunoassay data has been developed. Radioisotope counts transferred to punch-cards are automatically converted to a semilog plot of standard values from which assay values are derived and "printed out" as microunits of insulin and/or millimicrograms of growth hormone per milliliter of serum or plasma. *DIABETES* 16:734-37, October, 1967.

Radioimmunoassay methods for the measurement of protein hormones have become important technics in medical research and diagnosis. Among the various approaches used, the two-antibody method has gained wide acceptance. Considerable experience has been gained with two-antibody immunoassay of insulin and growth hormone (assayed individually or simultaneously), and the method has been simplified without sacrifice of precision so that serum samples in great numbers can be assayed by this procedure. In our own laboratory, several hundred serum or plasma samples have been assayed per week during the past year.

Processing of radioisotope counts and conversion of these numbers into standard curves and hormone concentration values had become a limiting factor. Accordingly, we have developed a computer program to alleviate this problem.

MATERIALS AND METHODS

The two-antibody method with recent modifications has been the radioimmunoassay utilized in these experiments.¹ The Packard Auto-gamma Spectrometer* with dual channels has been used for counting the radioisotope samples, Fortran IV programming language and an IBM 7040 computer have been used for processing the data.

RESULTS

The essentials of the program developed for simultaneous insulin-growth hormone assay are as follows:

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Routinely we use ninety tubes in an "assay set" so that all of the tubes in the set will be (1) incubated for the same time at the same temperature, (2) centrifuged at the same time at the same temperature, and (3) counted within three hours, thus obviating any correction factor for the relatively short half-life of I-131 when only precipitates are counted.

The first twenty-eight tubes of each "assay set" contain the standards; tubes 29 to 90 contain the assay samples. Tubes 1 to 4 contain buffer solution, i.e., "0" standard; tubes 5 to 7 contain either "1" μ U. of insulin or 1 $m\mu$ g. of growth hormone or both; tubes 8 to 10 contain "2"; tubes 11 to 13 contain "4"; tubes 26 to 28 contain 128. With assay of 0.5 ml. of serum or plasma, a potential standard range of "0" to "256" per milliliter is obtained.

The digits from the read-out paper tape of the radioisotope counter are transferred to punch cards.† The data cards with the appropriate program are then "fed" to the computer.

The computer program is divided into three sections: (1) derivation of standard curves, (2) computation of values for samples, and (3) control. The program is a combination of the programs for the individual assays of insulin and growth hormone. The computations for the two hormones are separate except that to obtain net I-125 counts for each tube, the gross I-125 counts are reduced by the background counts and by the percentage of I-131 counts that overlapped the I-125 spectral setting (25 to 73 KEV) as previously described.¹

The standard curve routines for insulin and growth hormone are similar. The mean and standard deviation of cpm in the precipitate for each concentration are computed. A linear function of the log of the concentration is fitted by least squares to these means. The "0" standard is not used for this fit as log 0 is undefined.

Since the standard curves are not always linear throughout the range of 0 to 128, the least square fit is determined from only the linear portion of the curve. To avoid errors from extrapolation beyond the limits of linearity, samples out of the range of these concentrations are not given definite values but are described as "greater than" or "less than" the end-

*Packard Instruments, Downers Grove, Illinois.

†The Packard counter can be adapted to read-out data on punch-tape, magnetic tape, or punch-cards for an appropriate fee.

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SIMULTANEOUS ASSAY 1-17-67 C

COUNTS PER MINUTE VS. LOG MICROUNITS SERUM INSULIN

ONE UNIT ON THE X SCALE = 0.213E-01

ONE UNIT ON THE Y SCALE = 0.249E 03

= INDICATES THE MORE THAN ONE POINT IS PLOTTED IN THAT SPACE.

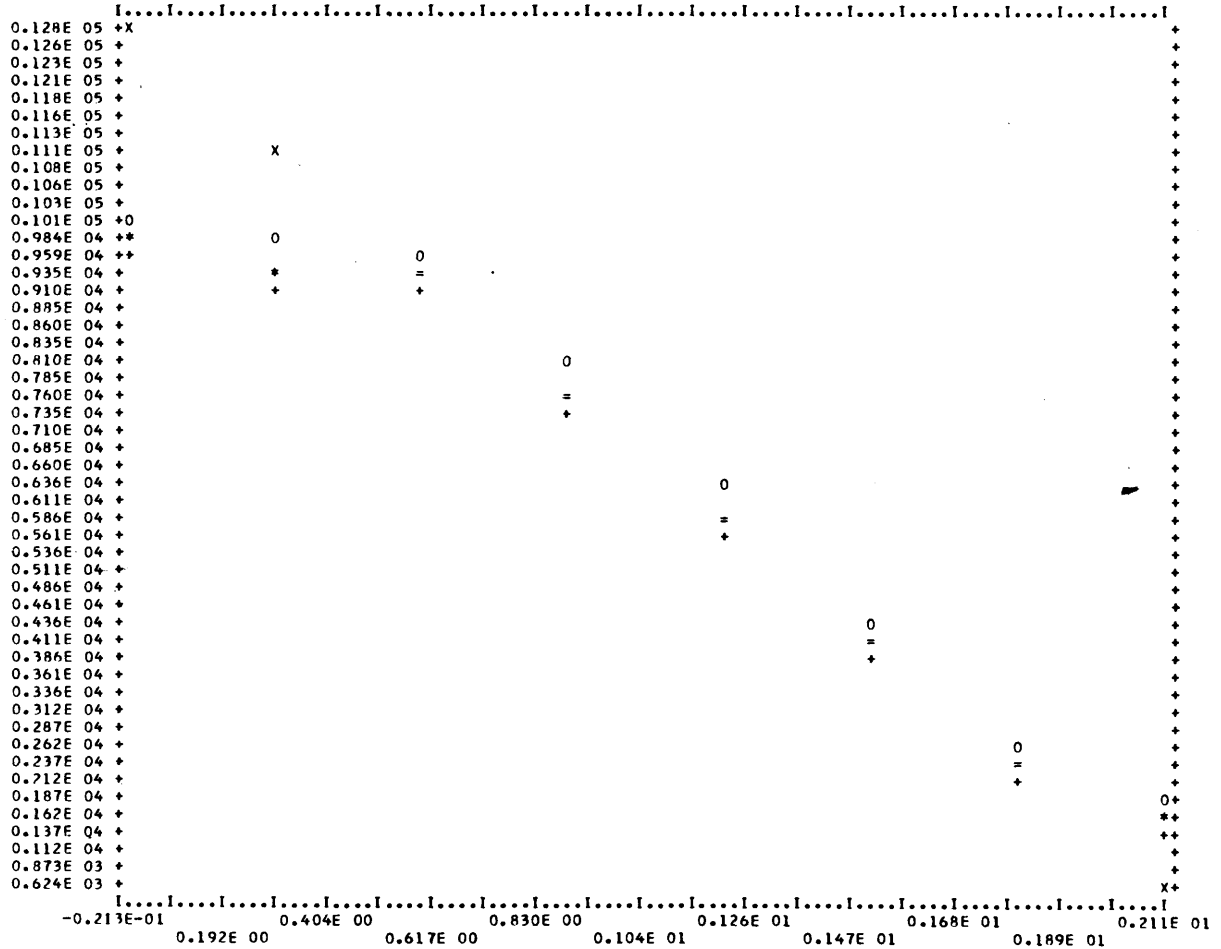


FIG. 1. The computer plot of the standard curves for insulin and growth hormone. The counts per minute of radioisotopes are on the "y" axes. The numbers are presented in exponential form, e.g.: 0.128E 05=12,800 cpm, 0.624E 03=6,240 cpm. The log of the hormone concentrations are plotted on the "x" axes in exponential form. The plot symbols are:

- * represents computed mean of 3 samples at each concentration.
- 0 represents mean plus one standard deviation.
- + represents mean minus one standard deviation.
- X represents least square fit.
- = represents more than one of above values plotted at the same point.

points of linearity. For both insulin and growth hormone, plots are made of the means, one standard deviation about the means, and the linear least squares fit by a "plotting sub-routine."

The fitted curves which are in slope-intercept form are transposed to make the serum hormone concentrations functions of the counts per minute of I-125 and I-131. The net cpm are "plugged" into their respective equations and each serum hormone concentration is printed to the right of the appropriate tube number.

The control section avoids specifying the number of assay sets and the number of samples in a "set." It tests for the end of a "set" and the end of a "run."

DISCUSSION

The computer program described here has been of great usefulness in processing two-antibody radioimmunoassay data in our laboratory. We feel it may be of use to others as well. Minor modifications to handle other quantities of serum or tissue extracts can easily be made.

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SIMULTANEOUS ASSAY 1-17-67 C

ASTERISK(S) AND PLUS SIGN(S) INDICATE EXTRAPOLATED VALUES.

* READ AS LESS THAN 8.0

** READ AS GREATER THAN 128.0

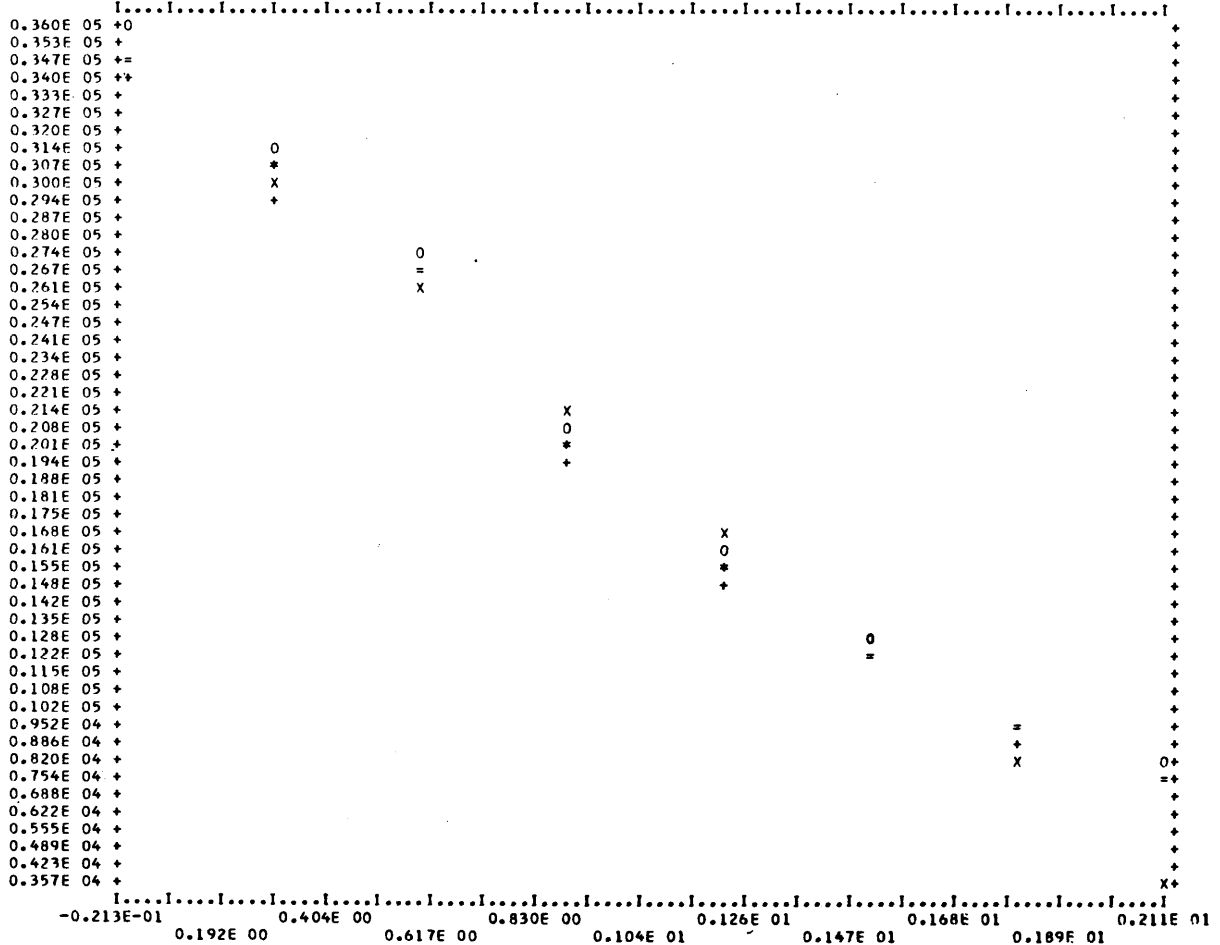
+ READ AS LESS THAN 2.0

++ READ AS GREATER THAN 128.0

PATIENT NUMBER	I131 COUNTS PER MINUTE	I125 COUNTS PER MINUTE	MICROUNITS OF INSULIN PER ML.	MILLIMICROGRAMS OF HGH PER ML.
29	7790.	31366.	14.84	3.37
30	7883.	31220.	14.30	3.45
31	9537.	28644.	7.41 *	5.15
32	9493.	25779.	7.54 *	8.05
33	9644.	31824.	7.10 *	3.14
34	9664.	32434.	7.05 *	2.86
35	9333.	21698.	8.04	15.21
36	9490.	23755.	7.55 *	11.04
37	4566.	28047.	53.44	5.66
38	4255.	27609.	60.47	6.06
39	4561.	28740.	53.55	5.08
40	4682.	29684.	51.04	4.38
41	6387.	24958.	25.92	9.15
42	6033.	24789.	29.83	9.40
43	9705.	24989.	6.93 *	9.11
44	9358.	24752.	7.96 *	9.45
45	9545.	25107.	7.39 *	8.94
46	9928.	27918.	6.35 *	5.77
47	9721.	27972.	6.89 *	5.72
48	9521.	26414.	7.46 *	7.30
49	8625.	26903.	10.65	6.76
50	8394.	25878.	11.67	7.93
51	5836.	28057.	32.26	5.65
52	5446.	26699.	37.67	6.98
53	7182.	27769.	18.90	5.91
54	6965.	29495.	20.60	4.52
55	7662.	28563.	15.62	5.22
56	7626.	29919.	15.84	4.23
57	7510.	28454.	16.59	5.31
58	7656.	28727.	15.65	5.09
59	8276.	28016.	12.24	5.68
60	7824.	27641.	14.64	6.03
61	10035.	31250.	6.08 *	3.44
62	10198.	28772.	5.70 *	5.05
63	2504.	26411.	121.28	7.30
64	2609.	26846.	116.32	6.82
65	2731.	26185.	110.81	7.56
66	2878.	26472.	104.53	7.23
67	3212.	25811.	91.53	8.01
68	3119.	23445.	94.98	11.58
69	2290.	19694.	132.04 **	20.78
70	2656.	20327.	114.17	18.83
71	2577.	20030.	117.81	19.72
72	2742.	21725.	110.33	15.14
73	2219.	24410.	135.82 **	9.97
74	2243.	23921.	134.53 **	10.76
75	4449.	24707.	55.99	9.52
76	4951.	27686.	45.86	5.98
77	4966.	30025.	45.59	4.16
78	4861.	28843.	47.53	5.00
79	5151.	17065.	42.36	31.29
80	4893.	16972.	46.93	31.75
81	5447.	18201.	37.66	26.22
82	4805.	14636.	48.60	45.68
83	4346.	14584.	58.33	46.05
84	4209.	14195.	61.59	48.93
85	4968.	16194.	45.55	35.84
86	5189.	18337.	41.72	25.67
87	9172.	22211.	8.57	14.04
88	9318.	22210.	8.09	14.04
89	4356.	25159.	58.10	8.87
90	4569.	25884.	53.38	7.92

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SIMULTANEOUS ASSAY 1-17-67 C
 I131 BACKGROUND= 200.0 I125 BACKGROUND= 120.0 PERCENT=0.190
 COUNTS PER MINUTE VS. LOG MILLIMICROGRAMS SERUM HGH
 ONE UNIT ON THE X SCALE = 0.213E-01 ONE UNIT ON THE Y SCALE = 0.662E 03
 = INDICATES THE MORE THAN ONE POINT IS PLOTTED IN THAT SPACE.



SLOPE= -0.14783922E 05 INTERCEPT= 0.34722678E 05
 COUNTS PER MINUTE OF 0.0 MILLIMICROGRAMS SERUM HGH = 37492.60 S.O.= 2439.05

FIG. 1. (Continued): The computer plot of the standard curves for insulin and growth hormone. The counts per minute of radioisotopes are the "y" axes. The numbers are presented in exponential form, e.g., 0.128E 05=12,800 cpm, 0.624E 03=6,240 cpm. The log of the hormone concentrations are plotted on the "x" axes in exponential form. The plot symbols are:
 * represents computed mean of 3 samples of each concentration.
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REFERENCES

¹ Morgan, C. R.: Immunoassay of human insulin and growth hormone simultaneously using I-131 and I-125 tracers. Proc. Soc. Exp. Biol. Med. 123:230-33, 1966.