

As for immunoreactive insulin assay, pooled serum, NMS-I and NMS-II were measured successively in each assay more than fifty times. Within-assay variability of reference sera were all below 10%. Between-assay variabilities showed coefficient variation below 15%. There was no large difference in coefficient variation among pooled serum, NMS-I and NMS-II.

Other assays including T-3, T3RU, TSH, FSH, HPR, HGH, Gastrin, Cortisol, IgE, AFP and others showed satisfactory reproducibility within the range allowed as between-assay variability of radioimmunoassay. However there were significant differences between indicated values and values obtained by us, except HGH, FSH and Gigoxin

of NMS-II. LH was not able to be measured because of very high value. Immunoreactive insulin was measured using three different kits. The values obtained were significantly different each other.

Therefor one of main reasons of difference between obtained values and indicated values was considered to be probably due to different assay system used. However, these control sera are maintained within permissible range as far as reproducibility is concerned, so that we could use them for the control of reproducibility of routine assay system. HB_sAG was always negative by sensitive radioimmunoassay.

Functions for Computation of Best Fitting Standard Curve in Radioimmunoassay of Hormones

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In order to evaluate the functions for computerized standard curve calculation for RIA of hormones, interassay variations of standard hormone levels in 5 assays were determined using calculator YHP MODEL 30 (4kW) and HITAC-20 (32 kW).

The standard curves were linearized by YHP MODEL 30 using raw, logit, arcsine or probit for B/B_0 on vertical axis and logarithm of hormone concentration on horizontal axis. The interassay variations were also calculated by HITAC using Rodbard's best fitting function (RBFF) program. The hormones examined included TSH, LH, FSH and IRI by double antibody, T₃ by dextran coated charcoal method and cortisol by polyethyleneglycol method. RBFF turned out to show significantly

smaller variation than those of other 4 functions especially in the range of high or low concentration of hormones. Among the 4 functions for linearization, logit gave the least error for TSH, FSH, T₃ and C-AMP while probit for cortisol, LH and IRI. The manual drawing of the standard curve by inspection resulted in the range of .30 to .90 of B/B_0 . It is concluded that linearization of standard curve by logit or probit transformation is practically useful in automatic calculation of hormone levels especially in the range of .30 to .90 of B/B_0 . RBFF covers, however, much wider range of hormone concentrations with smaller interassay variations establishing the validity of large computer system.

Determination of TSH by Simultaneous Equations

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The currently available Competitive Radioassay has been shown to have some defects. Thus we

have tried to reform this current method of Competitive Radioassay, applying it to the determi-

nation of TSH.

(THEORY)

As a calculation formula, the following one (1) is employed.

$$y = \frac{a}{x - c} - b \quad \dots\dots\dots(1)$$

y : Concentration of TSH $x = B/B_0$

The parameters (a , b , c) of the formula (1) are calculated according to the following simultaneous equations.

$$\begin{cases} 1.25 = \frac{a}{x_1 - c} - b \\ 20.0 = \frac{a}{x_2 - c} - b \\ 320 = \frac{a}{x_3 - c} - b \end{cases}$$

x_1 , x_2 , x_3 ; $B/B_0\%$ in each of the following concentrations (1.25, 20, 320 $\mu\text{u/ml}$) When the parameters (a , b , c) are calculated, the concentration of TSH can be determined according to the formula

(1).

(RESULTS)

(I) The correlation coefficient between the reformed assay system and the current method of Competitive Radioassay.

$$r = 0.999 \quad y = 1.03x - 1.4$$

(II) The interassay variations

$$n=10 \quad \bar{x}=7.47 \quad SD=0.95 \quad CV=12.8\%$$

$$n=10 \quad \bar{x}=50.8 \quad SD=3.98 \quad CV=7.8\%$$

$$n=10 \quad \bar{x}=129.5 \quad SD=7.81 \quad CV=6.0\%$$

(III) Advantages of the reformed assay system over the current method.

- 1) No necessity to draw the standard curve.
- 2) Simplicity in handling an electronic calculator and in dilution techniques of standard.
- 3) This method requires only 14 test tubes for the standard, while the current method does as many as 20 test tubes.
- 4) This method is less steps in calculator program than the other methods.

Theoretical Analysis and Data Processing of Competitive Radioassay

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In this report, a theoretical analysis of Radio-immunoassay among a variety of Competitive Radioassay is done.

This work is based on the assumption that the Antigen-Antibody Reaction is irreversible and the reaction is completed and the antigen is excessive over the antibody at the time of the completion of the reaction.

$$\begin{array}{ccc} (x=B/T) & (x=B/B_0) & (x=F/T) \\ y = \frac{A}{x} - b & y = \frac{b}{x} - b & y = \frac{-A}{x} - b \end{array}$$

y : Antigen in serum b : Labeled Antigen

A : Antibody

The correction of these formulas above described results in the following formula (1).

$$y = \frac{a}{x - c} - b \quad \dots\dots\dots(1)$$

The parameters (a , b , c) of the formula (1) can be calculated by the following simultaneous equations.

$$\begin{cases} y_1 = \frac{a}{x_1 - c} - b \\ y_2 = \frac{a}{x_2 - c} - b \\ y_3 = \frac{a}{x_3 - c} - b \end{cases}$$

Consequently, the quantity of antigen in the sample serum (y) can be calculated by the formula (1).