LETTERS TO THE EDITOR

Re: Evaluation and Comparison of Two Fully Automated Radioassay Systems with Distinctly Different Modes of Analysis

Concerning the article by I. W. Chen et al. (*1*), which I read with much interest, I make the following remarks.

From Table 1, which shows the intra- and interassay precision at different concentrations, it appears that the interassay variability is often less than the intra-assay variability. This finding is rather puzzling. Indeed, as explained by D. Rodbard (*2*), the observed interassay variance, S^2, equals

$$S^2 = S_b^2 + S_w^2/r, \quad (1)$$

where S_b^2 would be the "intrinsic" component of the interassay variance if we had an infinite number of replicates in each assay; S_w^2 is the component of variation due to measurement error within an assay for a single tube (i.e., the intra-assay variance); and r is the number of replicates in each assay.

Since the intra-assay variance bears on the assay of a single tube, it is advisable to express the interassay variance in the same way. It suffices to solve for S_b^2 in Eq. 1, where S^2, S_w^2, and r are known, and to recompute S^2 for r = 1 using the same equation. Otherwise, when quantifying the interassay variability by the variance of the assay means, as I presume was done here, one must state the number of replicates in each run lest the resulting figure be meaningless. For example, let us suppose that the results bear on ten replicates per run. Without any change in the variability of the system, and computing the coefficient of variation (CV%) the way it is supposedly done, the use of only two duplicates in each run should result in a figure of about 6.23% CV for the interassay variability, and even of 8.32% CV if r = 1.

It is regrettable in a study comparing the performances of assay systems, where precision deserves most careful attention, that the method and computations used are not presented. If, by chance, the number of replicates used with the different assay systems (batch, sequential, and manual) was not the same, this would invalidate the comparison as far as interassay precision is concerned.

Using the method described by Rodbard (*2*), we measured the precision of the T_4 assay on the sequential system.* Commercial control sera† at three dose levels were assayed in triplicate in ten runs. The results are expressed as the coefficient of variation (%) to be expected for r = 1 (Table 1).

As can be seen, the intra-assay precision is in agreement with the results in the article, whereas the interassay precision is clearly much less.

TABLE 1. PRECISION EXPRESSED AS WITHIN-ASSAY (INTRA) AND BETWEEN-ASSAY (INTER) COEFFICIENTS OF VARIATION

Level μg/dl	CV % (r = 1) Intra	Inter
4.0	7.8	12.0
7.6	4.6	8.6
19.0	5.6	8.2

FOOTNOTES

* Aria II, Becton Dickinson Laboratory Systems.
† Hyland Diagnostics, Division of Travenol Laboratories, Inc.

P. DEVOS
University of Leuven
Medical School
Leuven, Belgium

REFERENCES

1. CHEN IW, MAXON HR, HEMINGER LA, et al: Evaluation and comparison of two fully automated radioassay systems with distinctly different modes of analysis. *J Nucl Med* 21:1162-1168, 1980
2. RODBARD D: Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. *Clin Chem* 20:1255-1270, 1974

Reply

Dr. Devos has commented on our paper dealing with the analysis of precision in automated radioimmunoassay systems (*1*). The percent coefficients of variation are given for duplicates within assays (CVw) and for singles between assays (CVb). Coefficients of variation were determined by analyzing each control sample in duplicate in each of several assays in all three systems studied. The variance between analyses was calculated according to standard analysis of variance without determining the components of variance of means (*2*). We prefer to estimate the total variance of means (variance of means alone plus variance of measurement error) when determining whether the variance between means is greater than that of analysis despite a limited number of replicates. Accordingly, the CVb reported in our paper represents the variance for means of duplicates, whereas CVw is for the single tube.

A CVw greater than CVb is unusual but may occur when CVb is small, as in the case of the T_4 assay in the sequential system, or when CVw is large because the number of replicates assayed in each run is small. The conditions of the assays in question lead to these results. We used duplicates in our studies instead of higher replication since the precision of the assay warrants this, and because we wanted to evaluate the precision of the automated radioassay systems under conditions as close to the routine laboratory conditions as possible. The CVw listed in Table 1 of our paper would apply to a single measurement of an unknown falling in the same general region on the dose-response curve. If the unknowns are analyzed in duplicate, as is the case in our laboratory, the values of CVw should be divided by $\sqrt{2}$. We, however, customarily express our results in terms of singles. In our laboratory different numbers of replicates may be used, and expression of CVw for singles provides a satisfactory estimate of the reproducibility of the assay for our purposes.

We have since accumulated more data on the sequential automated system, as shown in the accompanying table. Unfortunately, we no longer keep the other two systems in our laboratory, and thus