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Computerized Quality Control of Radioimmunoassay in Korea

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Automated data processing and quality control of radioimmunoassays offer not only increased speed but also a more thorough and statistically rigorous analysis of results. An external quality assessment scheme for serum thyroxine, triiodothyronine and thyroid stimulating hormone (TSH) assays was performed in five nuclear medicine laboratories in Korea to compare with the assay performances of the World Health Organization Radioimmunoassay Program. The required radioimmunoassay kits were supplied through the International Atomic Energy Agency (IAEA). We have determined the weighted root mean squared error, and variance ratio as the indices of standard curve and also the average batch coefficient of variation (ABCV) as the parameters of response error relationship curve and precision profile. There was a good fit for the triiodothyronine assay, but 3 of 5 laboratories showed possible bad fit in the T4 and TSH assay systems. The ABCV was less than 5 percent for the T3 and T4 assay system, however for the TSH system, only 1 laboratory showed the ABCV value of less than 5 percent. We have also calculated the within batch variation (drift) and between laboratory variations.

Key Words: *Radioimmunoassay, quality control, computerized*

INTRODUCTION

After the invention of the radioimmunoassay (RIA) by Berson and Yalow in 1960, it has been used widely in the medicine due to its high sensitivity, specificity, simplicity and applicability (Edwards, 1985). With the development of such sensitive assay system, it became possible to measure the very small amounts in picogram, nanogram levels of analytes. So the assay system has been used in the fields of endocrinology, hematology, oncology, and infectious disease, etc.

But RIA is also subject to many errors. Some of these errors arise from the laboratory manipulations: i.e., pipetting error and separation error. And some errors also arise from the avoidable counting and statistical error. So, the objectives in the analysis of RIA data should be to determine the precise concentration of the analyte in each sample, to estimate the reliability of these results, and to assess the consistency of performance of the assay procedures over time.

Good data processing and quality control deal with all of these issues. But such analysis is too tedious to perform manually in routine work, and represents a reason for automation of data processing.

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The authors tried not only to perform automatic data processing but also to establish quality control among several laboratories in Korea and compared with the results of WHO RIA Program. The required assay kits were supplied through the International Atomic Energy Agency (IAEA).

MATERIAL AND METHOD

Five medical laboratories participated in this project. Among the many RIA systems, we chose T3, T4 and TSH assay system because these analytes are measured most widely in Korea. These RIA systems of thyroid hormones were composed of reagents supplied by IAEA. Polyethylene glycol (PEG)-second antibody separation technique was used in the T3 and T4 assay systems, and immunoradiometric assay (IRMA) technique was used for the TSH assay system. As quality control (QC) pool sera, samples collected from patients in Department of Nuclear Medicine, Seoul National University Hospital were distributed to various laboratories. We prepared 3 pools of sera for each particular thyroid function such as hyper, eu; and hypothyroidism, respectively.

In accordance with the WHO Immunoassay Program, with kits which was supplied by IAEA, we have measured weighted root mean squared error and variance ratio as the indices of standard curve, average batch coefficient of variation (ABCV), parameters of response error relationship (RER) curve, and precision profile of obtained result. Also we calculated the within batch variation (drift) and the variation between laboratories. With the T4 data obtained at the Seoul National University Hospital, we have evaluated the between batch variation, and have drawn Shewhart chart and CUSUM chart to analyze the quality control serially.

We obtained all of these parameters utilizing a 16 bit IBM compatible computer system. The method used by the computer to perform these calculations is summarized below. The first step in preparing the standard curve is to make curve-fitting and the best fit line is the one that goes closest to all the points. In a "weighted" fit, the line is forced closest to those points that have the smallest error bars. The distance between the points and the fitted curve is shown as Di, and the standard deviation of the mean (SDM) of the points as SDMi. The best fit to the points is given by that straight line for which the sum of these squared ratios for all points is smallest, that is, $\Sigma\ (Di/SDMi)^2$ is a minimum. Weighted root mean squared eorror was calculated by the equation of $1/n\ \Sigma\ (Di/SDMi)^2$.

Variance ratio test quantifies the goodness of fit of the fitted standard curve to the standards points. More exactly, it reveals if there is statistical evidence that the model for the curve is inconsistent with the data. The values of the standards points scatter about the fitted curve. The variance ratio test is an assessment of the probability (p) that the observed scatter in the replicates underlying each standard point could cause the means of the standards to scatter as much as observed with respect to the fitted curve, even if the population means for each standard were exactly on the fitted curve. If the probability is low (perhaps $p<0.3\%$), apparently there is very likely an inconsistency.

An alternative method of estimating within batch imprecision is by determination of the average batch coefficient of variation (ABCV), taking into account the responses from all samples in the batch, from standards, unknowns, and QC specimens. This value is calculated from the response error relationship (RER) which is a plot relating the errors in the responses (counts) against the mean value. When these are plotted on linear scales, the result is a "snow storm" or scatter diagram of points through which a linear regression line forced through the zero point may be drawn. The slope of this line is taken to represent the ABCV.

Like the RER, the precision profile is a description of the random non-counting-statistics errors in an assay procedure. However, it displays them in terms of, not of count rate but rather of, analyte concentration, which is the end result of interest. This profile allows for the problem of variation of precision with dose and is calculated from the results of all samples in one assay batch. Both the working range and the sensitivity of an assay are best determined from the precision profile. The former is that range of doses which fall within an acceptable level of precision, say a CV of 10% and the sensitivity is the limit of detection which is the precision of determination at zero dose.

Drift means an apparent systematic trend in the analyte concentration that is deduced for identical specimens spaced at intervals along an assay batch. Drift can be assessed by comparing the variance between sets and between individual pairs of the QC specimens. The former would be greater, but, if the difference is too great, say by a factor of 2 or more, then the possibility of drift must be considered.

RESULTS

Table 1 shows the results of "weighted root mean squared error" and "variance ratio" for each assay system. As determined by the variance ratio, this demonstrated a good fit for the T3 assay, but 3 out of 5 laboratories have shown 'possible bad fit' for the T4 and TSH assay systems.

Table 1. Weighted Root Mean Squared Error and Variance Ratio for T3, T4 and TSH Assay Systems.

	Assay System		
	T3	T4	TSH
Wt RM Sq Error	0.67±0.27	2.13±2.31	3.30±2.79
Variance Ratio	1.53±1.04	26.4±50.2	33.5±48.3

Unit: Mean±S.D.

Table 2. Average Batch Coefficient of Variation for T3, T4 and TSH Assay Systems.

Laboratory	Assay System		
	T3	T4	TSH
A	3.00	3.40	4.70
B	2.12	3.32	8.68
C	2.60	3.44	6.51
D	2.46	4.02	5.27
E	2.44	4.74	12.61
Mean	2.52	3.78	7.55
SD	0.31	0.60	3.21

Unit: %

Average batch coefficient of variations (ABCV) were less than 5 percent for the T3 and T4 system. But for the TSH assay system, only 1 laboratory showed CV less than 5 percent (Table 2) indicating that the TSH assay results were highly variable.

Range of doses which had less than 10% coefficient variation (CV) was described in TAble 3. Par-

Table 3. Range of Dose which had less than 10% of CV

Loboratory	Assay System		
	T3 (n m/1)	T4 (n m/1)	TSH (uU/1)
A	1.31–12.0	12.53–168.58	2.96–60.0
B	1.09–12.0	12.53–157.74	2.96–60.0
C	1.09–12.0	7.91–157.74	2.96–60.0
D	1.31–12.0	19.86–250.0	4.14–60.0
E	0.91–12.0	12.53–157.74	8.08–60.0

Table 4. Within Batch Variation (Drift) for T3, T4 and TSH Assay System

Laboratory	Assay System		
	T3	T4	TSH
A	−3.15	−0.46	−2.5
B	8.64*	−3.04	30.53**
C	22.50**	13.07**	15.46
D	11.94**	−6.88	0.11
E	−7.11	10.87	9.83

Unit: %
* probably significant
** possibly significant

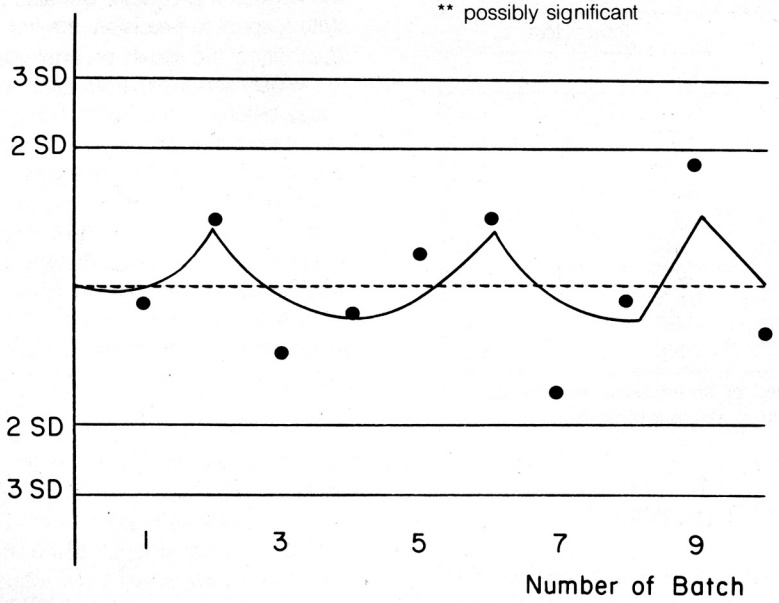

Fig. 1. Shewhart and CUSUM chart obtained from T4 assay

ticularly the TSH assay system showed unreliability in the measured concentrations at the lower level range.

Table 4 revealed the within batch drift. Laboratory A showed the stability in the intrabatch assay results, where as Laboratory C results showed unstability.

Overall variability between laboratories for the various QC pool is given in Table 5. Variability was marked particularly in samples where lower concentration of hormones was present. The T3 assay system revealed the least variation between laboratories.

Variation of parameter in RER curve for the T4 assay was demonstrated in Table 6. There were no marked changes after accumulation of a few batches.

Fig. 1 showed an example of Shewhart and CUSUM chart obtained in WHO Immunoassay Program.

Table 5. Between Laboratory Variation for T3, T4 and TSH Assay Systems

Assay System	QC Pool Name		
	Low	Med.	High
T3	17.8	8.7	5.6
T4	15.3	14.8	13.2
TSH	39.3	10.7	9.6

Unit: CV %

Table 6. Parameters of Response Error Relationship Curve obtained from T4 assay of Seoul National University Hospital.

Batch Number	Parameters	
	C_1	C_2
1	−0.032	0.033
2	−0.032	0.033
3	0.749	0.042
4	0.835	0.029
5	0.837	0.024
6	0.860	0.021
7	0.660	0.026
8	0.696	0.022
9	0.697	0.022
10	0.537	0.025

*Values were obtained by an equation desribed as: $y=C_1+C_2x$ (y: SDn, x: percent binding)

DISCUSSION

Variance ratio test quantifies the "goodness of fit" of the fitted standard curve to the standard points. More exactly, it reveals whether there is statistical evidence that the model for the curve is inconsistent with the data (Dudley et al., 1985). Weighted root mean squared error ($1/n \Sigma (Di/SDMi)^2$) is less informative than the true variance-ratio test, which also allows for the number of degree of freedom in each of the variances whose ratio is computed. In this result, T3 assay system showed the best fit among the tested thyroid hormone assay systems. Average batch coefficient variation (ABCV) shows precision of an assay system. It is a mean value of various CV according to the doses. ABCV values of less than 5% is regarded as good reproducibility. In our case, TSH assay system revealed the worst reliability, even though immunoradiometric assay (RIMA) method was used.

In this computer based data processing, noncounting statistical error was obtained by the curve of response error relationship (RES). The computer can deduce the equation of RER curve by automatic data analysis. In the T4 assay carried out at the Seoul National University Hospital, we can see the consistency of RER parameters, which means that the laboratory technique is stable.

The precision profile is the CV value against dose. It shows how the random errors in the measured value of dose. This is a key issue in evaluating the reliability of the analytical procedures, or in choosing bewteen one possible procedure to another. Usually, range of doses which had the CV less than 10% is satisfactory for routine RIA system (Rodbard, 1978).

QC pools provide specimens for testing not only the aspect of precision, but also the aspect of bias. With respect to precision, the first issue is the reproducibility of the results on equivalent specimens that are widely separated from each other within a single assay batch, i.e. the "drift". The second issue is that the obtained results should reveal low variations on equivalent specimens analyzed in different assay batch, i.e., the "between batch variation" (McDonagh et al. 1977; Kemp et al., 1978; Thackur et al., 1985). In practice, the results of "between-batch variation" is also demonstrated with Shewhart or CUSUM chart. But it is very laborious and cumbersome to calculate these variations and draw charts manually. In this computer program, we can do these works within 10 minutes.

Besides the use of this internal quality control, inclusion of external quality control is also highly advantageous. In this system, some external organization sends specimens to each of many laboratories and analyses the consistency of the obtained results are reported. However, without adequate internal quality control, a laboratory will be unable to take informed corrective measures in response to a report from an

external schemes that its results are unreliable (Bahk et al., 1987) So, after the establishment of internal quality control with this program, we should make progress to the external quality control. With the "WHO Immunoassay Program", we evaluted the quality of laboratories in our country. Continuous efforts for improving the reliability of RIA, such as regular internal and external QC, workshop, etc are needed.

REFERENCES

Bahk YW, Kim WI, Chung SK: *Progress report on external quality assessment in radioimmunoassay of thyroid-related hormones in the Republic of Korea. 1986. Korean J Nucl Med 21:1-4, 1987.*

Dudley RA, Edwards P, Ekins RP, Finney DJ, McKenzie IGM, Raab GM, Rodbard D, and Rodgers RPC.: *Guidelines for immunoassay data processing. Clin Chemist 31: 1264-1270, 1985.*

Edwards R: *Immunoassay. An introduction, London, William Heinemann Medical Books. pp12-23, 1985.*

Kemp KW, Nix ABJ, Wilson DW, Gaskell SJ: *Internal quality control of radioimmunoassay. J Endocrinol 76:203-210, 1978.*

McDonagh BF, Munson PJ, Rodbard D: *A computerised approach to statistical quality control for radioimmunoassays in the clinical chemical laboratory. Computer Progr Biomed 7:179-185, 1977.*

Rodbard D.: *Statistical estimation of the minimal detectable concentration for radioligand assays. Annal Biochem 90:13-21, 1978.*

Thackur AK, Listwak SJ, Rodbard D.: *Quality Control for Radioimmunoassay. Int. Conf. on Radiopharmaceuticals and Labelled Compounds. International Atomic Energy Agency, Vienna, pp345-360, 1985.*