QUALITATIVE EVALUATION OF IN-HOUSE IMMUNOASSAYS OF T3, 14 AND TSH BASED ON BULK REAGENTS

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ABSTRACT

Increase in the cost of RIA kit assays has led to our attempts to seek cheaper alternates. Assays based on bulk reagents (supplied free of cost by INMOL in collaboration with IAEA) were started in 1988. Statistical and Quality control data on 50,51 and 52 assay batches of T3, 14 and TSH respectively has been collected from the beginning. Cumulative assay parameters show that 13 and T4 assays are almost equally precise. TSH assay is most imprecise in the group especially at low concentration levels. The working ranges of T3 and T4 assays defined at 10% error limit are quite wide and cover low, medium and high levels of hormones. In TSH the assay working range does not cover levels below 10u lU/mi. The variability of curve parameters is similar in this group of assays. Quality control results are most reproducible in 14 assays with a between batch variability of 11.9%. T3 and TSH assay results are equally reproducible (20.50% variability). Overall within assay drift is low in all assays. IQC charts of these assays show occasional significant positive or negative shift of results from mean which might be related to methodological variations of quality among various distributions of reagents. The reproducibility and precision of results could be further improved by harmonizing the future distributions of reagents (JPMA 41: 63, 1991).

INTRODUCTION

The scope of inimunoassays in developing world depends on availability of financial resources. The commercial kits are expensive and losing their popularity. The cost of running an in-house assay for either thyroxine or triiodothyronin with all ingredients purchased, to process 100000 assay tubes, would be around US$ 1000, whereas a commercial kit will cost US$ 9000[1]. Lowering the cost should not, however, result in the lowering of quality. This laboratory has developed a system of internal quality control (IQC) to evaluate validity of the assays before the release of results to the patients. A scheme on bulk supply of RIA reagents supported by International Atomic Energy Agency was started few years back at INMOL, Lahore. We were one of the participating laboratories of this scheme. In the very beginning of this scheme we decided to routinely evaluate the in-house assays in terms of quality and accumulate the relevant data to detect long term trends or defects. This paper elaborates our strategy of quality control as applied to in-house assays.

MATERIALS AND METHODS

In the beginning in-house assays were performed parallel to Amersham kit assays[2-4] to assess the correlation between the two techniques. Later on statistical and quality control data on in-house assays alone was regularly recorded. The assays of T3 and T4 involved double antibody precipitation. The first and second antibodies were supplied by NETRIA (North East Thames Region Immunoassay Unit U.K), through INMOL (Institute of Nuclear Medicine and Oncology, Lahore). In-house TSH assays used IRMA principles[5]. 1251-anti-TSH and Solid phase anti-TSH were prepared at INMOL, Lahore.

Other reagents were supplied by NETRIA. The whole scheme was supported by IAEA. The bulk supplies were not complete in all respects and included single reagents or a combination of two or three. The assay set up in each type was composed of duplicate tubes of total activity, non-specific binding, standard and unknown serum samples. A comprehensive design is presented in Table 1.

TABLE I. Assay Design (Sequence of Tubes) Adapted in Assays of Hormones.

	Nature of sample	Lebel used	Meaning
1.	Standards for calibrating the standard curve	-1	Background count tube
		0	Reference standard tube
		1,2	Total activity tubes
		3,4	Zero Concentration standard.
		5,6	Non-specific binding (NSB) tubes.
		7,8---N	Calibration standards (in duplicate)
		-1	Background count tube.
		0	Reference standard tube.
2.	Unknown or Patient samples.	-1	Background count cube
		0	Reference standard tube
		1-6	QC Pools (low,medium, high in duplicate).
		7-36	15 Unknown samples (in duplicate)
		37-42	QC Pools (low, medium, high)
		43-72	15 Unknown samples (in duplicate)
		73-78	QC Pools (low, medium, high).
		-1	Background count tube.
		0	References standard tube.

Quality Control pooled sera (low, medium, high) prepared at this centre, were placed in three groups in beginning, middle and end of unknown sample tubes. Test procedures were those supplied by NETRJA. All T3 and T4 assays were performed by one technician. TSH assays were done by another technician. Data analysis was done on IBM compatible computer using data processing programmes supplied by International Atomic Energy Agency. The philosophy and principles of these programmes were set out by Dudley RA[6-8] and their translation into computer programmes was done by Piyasena RD[9]. Assay Parameters i.e. RER Parameters, curve parameters, QC Pool results, % BBCV etc. were regularly recorded for comparison. Schewart IQC (internal quality control) plots for in-house assays were also plotted in terms of percent deviation of individual batch means populations mean vs assay number to see the long term trend in results. The warning and control limits were calculated from all pooled data. The overall random errors (non-counting statistics %CV, denoted by R in these programmes) are shown in table II.

RESULTS

The correlation studies showed linear correlation between results of in-house assays and those of Amersham assays. The regression equations were following:T3 Y = 0.2 + 0.95 X Where Y = in house RIA
X = Amerlex-M RIA T4 Y = 5 + 0.99 X Where Y = in-house RIA
X = Amerlex-M RIA TSH Y = 2 + 0.90 X Where Y = in-house IRMA
X = Amerlex-M RIA

Mean assay parameters along with between batch scatter (%CV) are given in table III.

TABLE III. Average assay Parameters With Between Batch Scatter Expressed as %CV Mean of Batch Values.

Analyte >	T3		T4		TSH	
Parameter	X	%CV	X	%CV	X	%CV
1. !RER Parameters						
A	1.2	125	1.000	94.3	3.5	38.0
B	0.02	159	0.035	164.8	-0.04	114.6
2. !Curve Parameters						
a	37.90	10.9	48.80	17.5	#63.60	2.3
b	0.94	9.5	0.96	10.0	-1.13	7.3
*c	1.96	49.2	28.7	25.1	121.80	37.5
d	1.9	12.9	1.3	38.1	0.43	25.6
Overall % BBCV		20.6		22.7		18.5
3.QC* Pool values						
Pool-1	0.66	27.2	16.3	17.3	3.56	30.20
Pool-2	1.78	17.3	116.8	8.4	12.83	19.64
Pool-3	4.42	22.6	203.1	12.3	19.01	15.10
composite	2.30	15.0	112.0	9.7	11.9	17.22
Overall %BBCV		20.5		11.3		20.50
Overall drift	1.3	340	0.43	568.0	-0.9	350.00

X=Mean of batch means.
*expressed in following units of concentration: T3 nmol/1, T4 nmol/1, TSH μIU/ml.
! A is intercept of RER Plot.
B is slope of RER plot.
a is intercept of logit-log standard curve.
b is slope of logit-log standard curve.
c is midplane dose at a+d/2
d is non-specific binding.
#Parameter "a" in TSH IRMA is based on assumed value i.e., 65[9]..

The average RER plots (solid line) of the assays alongwith the scatter of individual batch RER's (indicated by dotted line) arc shown in Figure 1.

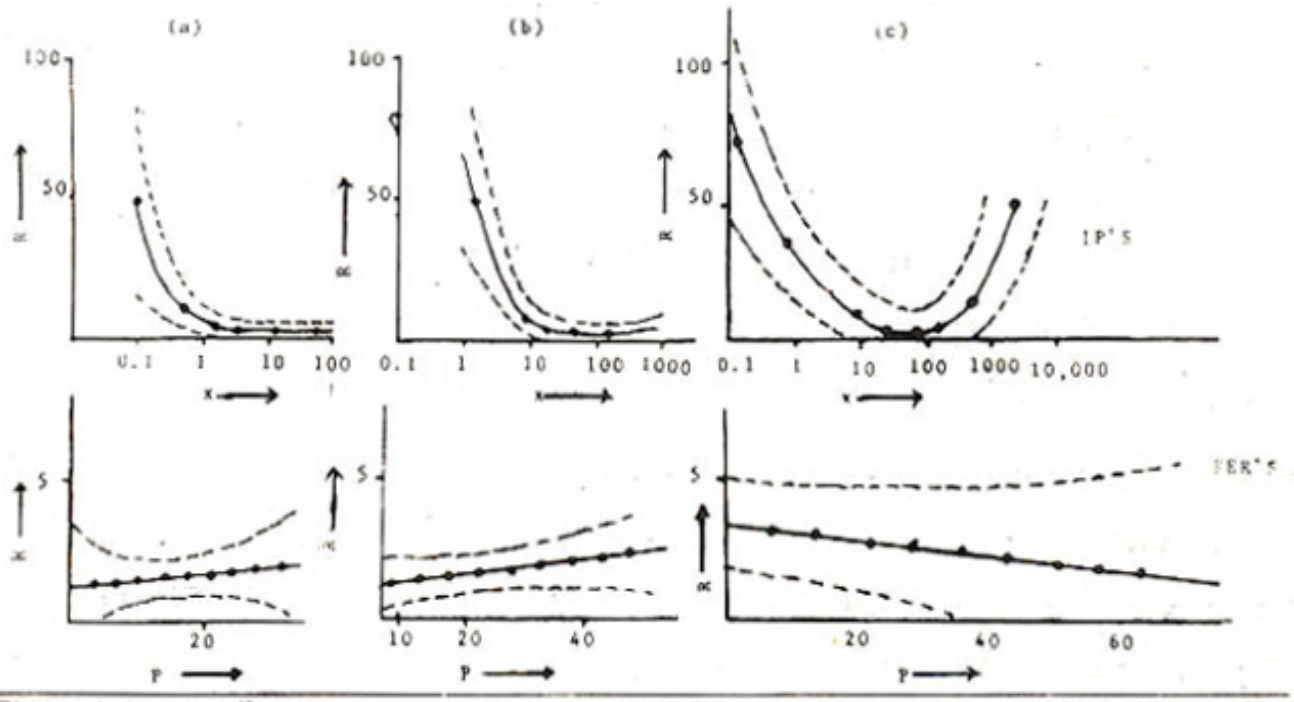

Figure 1.Average Response error relationships (RER's) and imprecision profiles (IP's) a) T3 RIA b) T4 RIA c) TSH RIA { average of 50 assays, ------ between Batch scatter (isd), x concentration of T3 or T4 in N. MOL/1, Xs Concentration of TSH in uIU/ml, P % B/T, R Non-counting statistics % CV].

The average imprecision profiles (solid line) and the scatter (dotted line) of individual batch IP\'s around the average profiles of the assays under discussion are also displayed in Figure 1.The errors within normal ranges and assay working ranges (defined at 10% error limit) as obtained from these profiles are given in Table II.

TABLE II. Within Assay Scatter (%CV of Replicate Counts) in Assays of each category (Period 1989-1990).

Analyte	Total no. of assay batches.	Standard Scatter		Unknown Scatter	
		Mean of batch means.	Range	Mean of batch means	Range
T3	50	0.99	0.0 – 5.1	2.08	0 – 5.1
T4	51	1.20	0.0 – 6.3	2.50	0 – 6.4
TSH	53	6.5	0.3 – 12.8	5.40	0 – 11.7

IQC charts are displayed in Figure 2.

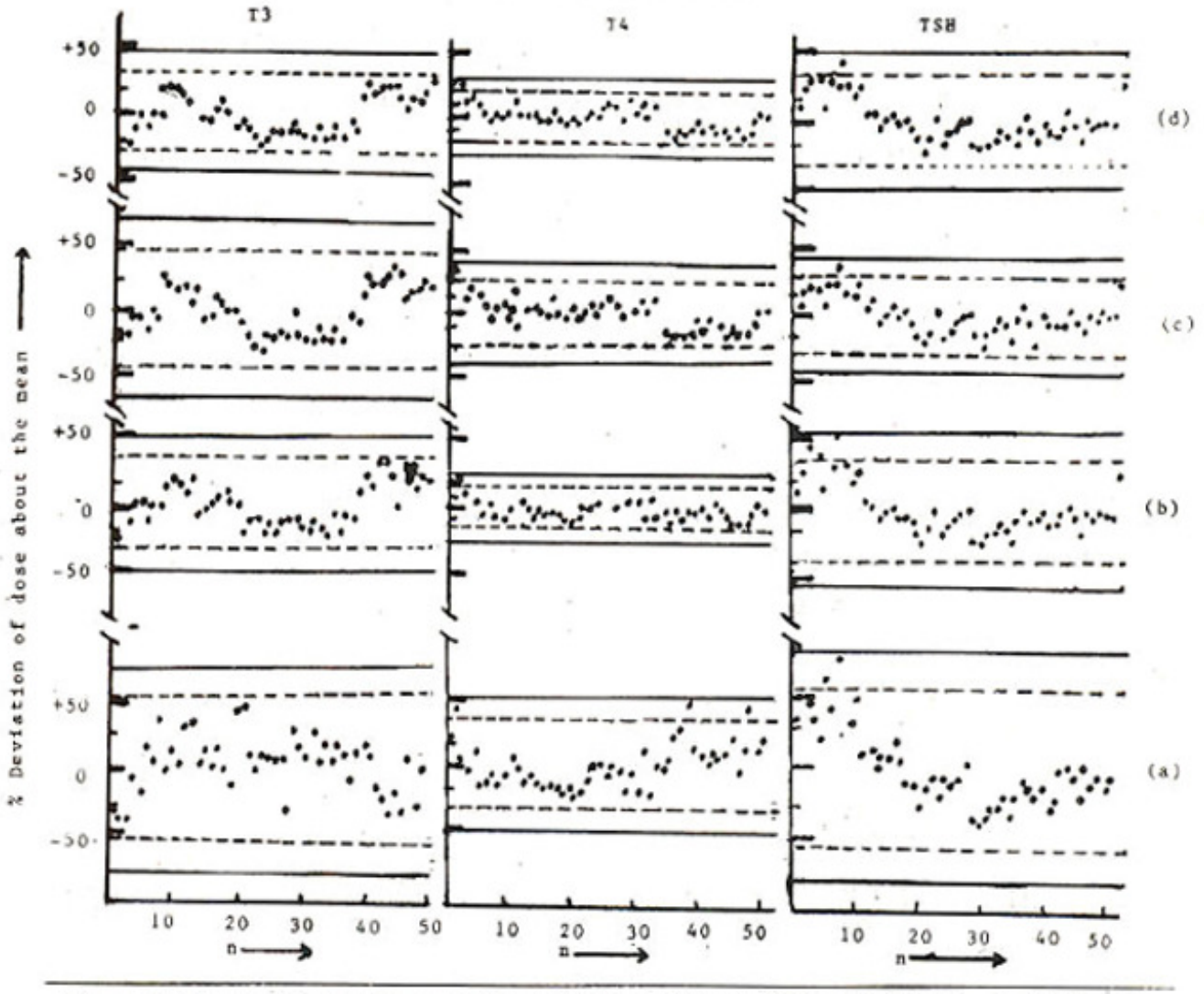

Figure 2 Schewart Quality Control Chart (a) Pool-1 (low) (b) Pool-2 (Medium), (c) Pool-3 (High), (d), Composite of three pools. [Control of limit (3SD), ----- Warning limit (2SD), n is number of assays performed].

DISCUSSION

Quality control procedures of an assay start when it is under designing stage and proceed through monitoring of results by IQC and end with retrospective analysis of IQC data to identify long term trends in performance[10]. The main objective is to get control over the entire assay and it's improvement[11]. Quality Control (QC) may be active or passive. Active QC concerns with modifications in assay set-up or procedure to improve characteristics like accuracy and ruggedness. Passive QC (or quality monitoring) concerns with the estimation of error in an assay. All potential sources of error cannot be examined and a real IQC programme is a compromise between the desire for complete knowledge and practical constraints, notably time, money and available personnel[12]. In our set-up Active QC was done at INMOL. Different batches of reagents were however checked to ensure that they are of acceptable quality. This is particularly important in double antibody methods, where different batches of same type of material may essentially differ in their working dilution and

vulnerability to serum effects[10]. The three main components of assay---standards, unknown and quality control samples give important information not only about the analyte concentration but also about the imprecision and bias errors inherent in these estimates. Manual handling of such information is very difficult and a need for automatic system is a necessity. Data reduction programmes in our use, offer a very comprehensive analysis of results[12]. The parameters which characterize assay performance are response error relationship (RER), imprecision profile (IP), curve parameters and spot QC sample results. A brief account of these parameters is following.

Response Error Relationship (RER) & Imprecision profile (IP).

Each individual specimen in an assay batch is analyzed in replicate, which facilitates the estimation of random error in measurements. A plot of error in response (P) versus response is obtained, which is known as response error relationship or RER[6-9,11-17]. Another plot which describes within assay imprecision is imprecision profile (IP), which is a plot of random error versus concentration. The IP is a useful tool for comparing an assay run with previous runs or for comparing different assay methods[18].

Curve fitting

Mathematical model for standard curve fitting offered in these programmes is called, the fitting of a 4-parameter logistic curve to the points, using weighted least square procedures. The equation giving rise to this curve contains 4 parameters, a.b,c, and d. A clear graphic technique embodied in programmes of Dudley[6-8] (a revised version of his programmes is in our use) and those of Malan[19] to assess the quality of standard curve plots the difference between known (true) and predicted (apparent) analyte concentrations of the standards, with predicted values bracketed by their confidence limits. This allows rapid check if the calibration method is appropriate for the assay at hand[12].

Spot QC sample

Spot Quality control samples are taken from a large pool of material which is carefully stored for long term use. A bit of material is analyzed in each assay batch to ascertain that the assay results are stable. Drift, the temporal shift of results within an assay run is assessed by spot quality control samples. A general plan for the disposition of spot quality control samples is offered by Ayers et al[20]. We have followed a three pool, three group pattern, as most experts recommend the use of three pools (low, medium, high concentrations) and introduction of a group of QC specimens (i.e., one specimen from each pool) at regular intervals (we have selected an interval of 30 tubes; Table-I[6-7]) Results obtained for the spot samples are important indices of quality when plotted on flow charts, because these can indicate any sudden shift in assay performance. The most commonly encountered type of chart is the simplest. Schewart of Levy- Jennings, type[12] CUSUM charts and other more complex varieties have found less favour[21].

Specific comments on results

The linear correlation of results of in-house assays with Amersham kits suggests that these can work as a replacement of commercial techniques giving parallel values. The overall random error (non-counting statistics % CV, denoted by R in these programmes) shown in Table-II is fairly low in T3 and T4 assays i.e., less than 7% in all assays (mean values less than 3%), indicating good overall precision of the assays. In TSH the levels of error are relatively high. The mean levels are, however, under acceptable 10% limits (i.e., less than 7%). TSH assay is therefore more imprecise relative to T3 and T4 assays. Response error relationship (RER), a powerful reflection of assay performance is differently calculated by different biostatisticians[12]. Malan and Dudley[7-8,15] employ a straight line fit to the standard deviation and coefficient of variation respectively. The programmes in our use relate non-counting % CV (R) with normalized mean counts or response (P). The parameters A (intercept) and B (slope) of the straight line thus obtained define overall non-counting errors in estimates of response in an assay batch. In our assay batches mean RER parameters (Table III), suggest an overall low level of random errors in the results. Although RER parameters scatter widely from batch to batch (indicated by %CV's in Table-

IV),

TABLE IV. Errors Observed Within Normal Range and Assay Working Ranges Defined by Average Precision Profiles at 10%CV.

Analyte	Units	Normal range	Error within normal range.	Assay working range (SI units).
T3	nmol/1	0.8–3.0	7.5–3.2	0.5 to > 50
T4	noml/1	58.0–174.0	2.5–2.6	7.0 to >1000
TSH	ulU/ml	1.0 –7.0	13.0–32.5	10.0 to >398

the individual RER's, enveloped by dotted line (which reflects the standard scatter of batch RER's around the average) in figure 1 do not cross the 10% error limit. This shows that random errors in our assays are not much affecting the accuracy of our results. Imprecision profile like RER is a description of random non- counting statistics errors in an assay procedure[7-9,14]. However it displays them in terms of analyte concentration (X) rather than response (P) and is dependent on the shape of standard curve and RER parameters. The average IP's of T3 and T4 assays (Figure I) in our set-up suggest an overall good and acceptable precision of measurements, whereas TSH assay seems less precise at lower levels and is precise at high dose levels. The scatter of individual batch IP's (shown by dotted line envelope in figure 1) is lowest in T3 and T4 RIA\'s and highest in TSH-RIA. Assay working ranges are quite wide in T3 and T4 assays to cover all clinical ranges. In TSH assay working range does not cover all clinical ranges. It excludes levels below 10 MIU/ml. All this indicates that TSI-1 assay is relatively imprecise and needs further improvement. Standard curve parameter 'a" (Table-III) is more reproducible in T3 as compared to T4 assay. This is shown by a between batch scatter of 10.9%. In TSFI the estimation of this parameter is based on assumed value i.e., 65 (recommended by programmer) [9]. Curve parameter "b', an estimation of slope of logitlog plot is dose to ideal value i.e., unity and appears to be the most stable parameter in assays of each category (indicated by %CV's equal or less than 10%). Parameter "C", scatters widely in all assays. It is, however most reproducible in T4 assay, where a between batch %CV of 25.1 is seen. Parameter "d", is most stable in T3 assay and scatters widely in T4 and TSH assays. However, in most assays the highest value is not more than 3%. The overall between batch variation of curve parameters is almost similar in all assays. This shows that curve parameters are almost equally reproducible in all assays. Results of Pool-i of QC spot samples indicate that these are least reproducible amongst all. This may be due to the fact that the concentration of hormones in these pools is low and wide variation may be expected due to high level of imprecision in low doses (see IP's in figure 1). A comparison of between batch CV\'s shows that T4 RIA is giving most reproducible results. T3 assay has intermediate reproducibility and TSH the least reproducible. In pool-2, T4 assay is again most reproducible and TSH the least. In pool-3 similar pattern is seen. Data on composite pool and overall between batch %CV reveals that T4 assay is most reproducible. T5 and TSFI assays are almost equally reproducible. Assays of each category show an overall low drift in results, although the individual assay values scatter widely from batch to batch. A comparison of IQC charts (Schewart) shows significant occasional positive and negative shifts in results which sometimes push the results to warning limits. These shifts are most probably related to the differences in quality among different lots and differences of reagents and could be removed by harmonizing different reagent supplies (active QC). This laboratory is in close contact with INMOL where such shifts are being scrutinized to relate them with quality of reagents. It is hoped that these will be washed very soon and rugged in-house assays will be feasible. To summarize, T4- RIA is very precise with comparatively highest reproducibility (or stability) of assay parameters (or results). T5- RIA is highly precise but with intermediate reproducibility. TSH-IRMA is the most imprecise with reproducibility comparable to T3 RIA. TSFI assay needs immediate attention to amend the assay set-up to improve the results in lower

levels. The occasional poor consistency in assay results as displayed by IQC charts is probably related to poor homogeneity of quality among different preparations, supplies and distributions. Active QC could minimize such trends.

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