

External Quality Assessment for radioimmunoassay of thyroid and related hormones: Third-year scheme.

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Background : *External Quality Assessment Schemes (EQAS) have been widely used in radioimmunoassay (RIA). The International Atomic Energy Agency (IAEA) launched a Regional Co-operative Project on EQAS for radioimmunoassay of thyroid related hormones for developing countries in Asia and Pacific Region. A total of 80 laboratories from 11 countries in this region including Thailand participated in the scheme. Satisfactory results were reported for the first and second year scheme. The present study is the third year scheme and only results from Bangkok Center will be reported.*

Objective : *The objective of this Regional Cooperative Project is to create interlaboratory surveys for performances on RIA of serum thyroxine (T₄), triiodothyronine (T₃) and thyroid stimulating hormone (TSH) in order to improve accuracy and reliability in their clinical services.*

Setting : *Division of Nuclear Medicine, Department of Radiology, Faculty of Medicine, Chulalongkorn University*

Research Design : *Prospective study*

- Material** : *Bangkok Center is one of the 3 designated centers of the scheme. Sixteen laboratories participated in the scheme under responsibility of Bangkok Center.*
- Methods** : *Three organizing centers prepared 8 EQA pools and randomly distributed 3 EQA samples each month for the whole year to participating laboratories. Participants were requested to assay T4, T3 and TSH in the EQA samples and returned their results batch by batch. Monthly and six-monthly results were analyzed and the results were returned to participants.*
- Result** : *The overall results of the present EQAS demonstrate considerable improvement in TSH assay performance as compared to previous scheme and further improvement is evidenced for T3 and T4 assay.*
- Conclusion** : *The present EQAS indicates further improvement in performance of radioimmunoassay in most participating laboratories.*
- Key words** : *External Quality Assessment, Radioimmunoassay, Thyroxine, Triiodothyronine, Thyroid stimulating hormone.*

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พวงพยอม ปรีชาภาส, มาคัมครอง โปษยะจินดา, ธวัชชัย ชัยวัฒน์รัตน. การประเมินคุณภาพของเรดิโออิมมูโนแอสเสย์ของทัยรอยด์ซอร์โมน และซอร์โมนที่เกี่ยวข้องโดยหน่วยงานภายนอก ในรอบปีที่ 3. จุฬาลงกรณ์เวชสาร 2540 ส.ค.;41(8): 575-91

- เหตุผลของการทำวิจัย :** ทบวงการพลังงานปรมาณูระหว่างประเทศ ได้จัดทำโครงการความร่วมมือทางวิชาการระหว่างประเทศที่กำลังพัฒนาในภูมิภาคเอเชียแปซิฟิก ในหัวข้อเรื่องการประเมินคุณภาพของการตรวจวัดทัยรอยด์ซอร์โมน และ ซอร์โมนที่เกี่ยวข้องด้วยวิธีเรดิโออิมมูโนแอสเสย์ ซึ่งมีห้องปฏิบัติการ 80 แห่งจาก 11 ประเทศในภูมิภาคแถบนี้เข้าร่วมโครงการ ซึ่งรวมทั้งประเทศไทยด้วย ผลการดำเนินโครงการรอบปีที่ 1 และรอบปีที่ 2 ได้ผลเป็นที่น่าพอใจ รายงานที่เสนอนี้เป็นผลงานในรอบปีที่ 3 และเป็นผลการศึกษาจากศูนย์ที่กรุงเทพฯ เท่านั้น
- วัตถุประสงค์ :** เพื่อสร้างวิธีการสำรวจคุณภาพของการตรวจวัดระดับซีรั่ม ทัยรอกซิน (T4), ไตรไอโอโดทัยโรนิน (T3) และซอร์โมนที่กระตุ้นต่อมทัยรอยด์ (TSH) เพื่อปรับปรุงความแม่นยำ และความเชื่อถือในงานบริการทางคลินิก
- สถานที่ที่ทำการศึกษา :** ห้องปฏิบัติการเรดิโออิมมูโนแอสเสย์ สาขาเวชศาสตร์นิวเคลียร์ ภาควิชารังสีวิทยา คณะแพทยศาสตร์จุฬาลงกรณ์มหาวิทยาลัย
- รูปแบบการวิจัย :** การศึกษาไปข้างหน้า
- การคัดเลือกผู้ป่วย :** ศูนย์กรุงเทพฯ เป็นหนึ่งใน 3 ศูนย์ ที่ได้รับการคัดเลือกในโครงการนี้มีห้องปฏิบัติการ 16 แห่งที่อยู่ภายใต้ความรับผิดชอบของศูนย์กรุงเทพฯ
- วิธีการทำวิจัย :** ศูนย์ปฏิบัติการทั้ง 3 แห่งร่วมกันเตรียมตัวอย่างสำหรับการทดสอบ 8 ชุด แล้วส่งให้ห้องปฏิบัติการต่างๆ แห่งละ 3 ชุด ทุกเดือน จนครบ 1 ปี โดยวิธีสุ่มตัวอย่าง ห้องปฏิบัติการเหล่านั้น ทำการตรวจวัดระดับของ T4, T3, และ TSH ในตัวอย่างที่ได้รับแล้วส่งผลกลับมายังศูนย์ปฏิบัติการทุกเดือน ศูนย์ปฏิบัติการวิเคราะห์ผลทุกเดือน และทุก 6 เดือน แล้วส่งรายงานกลับไปยังห้องปฏิบัติการต่างๆ เพื่อปรับปรุงแก้ไขต่อไป หากมีข้อบกพร่องจากการตรวจ
- ผลการศึกษา :** จากการศึกษาี้แสดงให้เห็นว่าการตรวจวัดระดับ TSH ของห้องปฏิบัติการต่างๆ ดีขึ้นอย่างมากเมื่อเปรียบเทียบกับผลการศึกษาในรอบปีที่แล้ว ส่วนการตรวจวัดระดับ T4 และ T3 ก็ดียิ่งขึ้นอีก
- สรุป :** จากการประเมินคุณภาพของการทำเรดิโออิมมูโนแอสเสย์ของห้องปฏิบัติการต่างๆ ใน รอบปีที่ 3 แสดงให้เห็นว่าส่วนใหญ่ มีการปรับปรุงคุณภาพของการตรวจวัดให้ดียิ่งขึ้นอีก

The External Quality Assessment Scheme (EQAS) has been widely used in radioimmunoassays.⁽¹⁻⁴⁾ The International Atomic Energy Agency (IAEA) launched a Regional Cooperative Project on EQAS for radioimmunoassays of thyroid related hormones for developing countries in the Asia and Pacific region in 1989. The objective of this project is to initiate interlaboratory surveys in this region for performance of radioimmunoassays (RIA) of thyroid and related hormones in order to stimulate interest in quality control and take remedial action for improving the performance of RIA which will result in increased accuracy and reliability of these clinical services. There are 11 participating countries and three organizing centers in Seoul, Lahore and Bangkok. Each center oversees 15-50 participating laboratories and there are about 80 laboratories participated in the whole scheme. Satisfactory results were reported for the first and second year schemes with evidence of significant benefit to the participating laboratories.^(5,6) The scheme described in this report is the third year scheme, and only the results from Bangkok Center will be presented.

Materials and Methods

Participating laboratories

There were 16 participating laboratories; 13 from Thailand, 2 from Sri Lanka and 1 from Myanmar. Malaysia and Vietnam had dropped out from this scheme for unknown reasons.

Outline of the scheme

The EQAS samples for distribution were prepared by the 3 organizing centers. For Bangkok Center, the external quality assessment (EQA) sera were obtained from blood donors who had negative tests for HBsAg, HCV and HIV. Eight pools were used in the scheme which comprised 4 simple pools and 4 manipulated pools. Recovery or manipulated pools were prepared by adding known amounts of T₄, T₃ and TSH. Bangkok Center prepared an euthyroid pool (pool 1) and a spiked T₄ pool (pool 2). The remaining 6 pools were prepared by the Seoul and Lahore Centers. Each center exchanged the EQA sera with the other centers for further distribution to their participating laboratories.

Participating laboratories received 3 EQA serum samples monthly for one year. They were asked to measure T₄, T₃ and TSH in each sample by their routine methods for clinical services. Results of the assays, together with internal quality control (IQC) results, were reported back to the organizing center monthly. Results from each distributed set were analysed and reported back to all participants. Cumulative results over each six-month period were also processed and reported.

Results

Distributed material

The distributed materials are shown in Table 1. All laboratories trimmed mean (ALTM)

were calculated after 10% trimming of the lowest and highest values and standard deviation was estimated by the method suggested by Heally.⁽⁷⁾

Reporting results

A monthly report, as shown in Fig 1, was sent to each individual laboratory. Laboratory number 7 uses FT4 for routine clinical service; therefore, no T4 result was obtained from that laboratory. Laboratories number 11 and 14 did not

perform TSH assays, in their routine services, hence there was no TSH result from these two laboratories. Laboratory number 6 provided very poor participation so the return result was only 26.5%. The overall rate of return from all laboratories was 89.5%

Cumulative statistics were calculated on 6-months of data and sent to participants. The results included mean bias and variability of bias (Table 2 and Table 3).

Table 1. Material distributed.

POOL IDENTITY	TRIMMED MEANS			NO.OF DISTRIBUTION
	T4 (nmol/L)	T3 (nmol/L)	TSH (mU/L)	
A. SIMPLE POOLS				
1. EUTHYROID	96	1.8	1.6	5
3. EUTHYROID	93	1.6	1.9	5
5. EUTHYROID	92	1.9	2.2	5
7. HORSE SERUM	19	0.7	0.3	2
B. MANIPULATED POOLS				
2. Pool 1 + T4 (20 nmol/L)	113	2.0	1.7	5
4. Pool 3 + T3 (1.5 nmol/L)	90	3.3	1.9	5
6. Pool 5 + TSH (10 mU/L)	90	2.0	13.1	5
8. Pool 7 + TSH (10 mU/L)	17	0.6	9.4	2

IAEA REGIONAL EXTERNAL QUALITY ASSESSMENT SCHEME
FOR THYROID RELATED HORMONES REGION : THAILAND

ANALYTE: T4 nMol/L

DISTRIBUTION		DEC 92					
LAB	SAMPLE 1		SAMPLE 2		SAMPLE 3		
1	0	-----	0	-----	0	-----	
2	-----0	-----	-----0	-----	-----0	-----	
3	-----0	-----	-----0	-----	-----0	-----	
4	-----0	-----	-----0	-----	-----0	-----	
5	-----0	-----	-----0	-----	-----0	-----	
6	-----	-----	-----	-----	-----	-----	
7	-----	-----	-----	-----	-----	-----	
8	-----0	-----	-----0	-----	-----0	-----	
9	-----0	-----	-----0	-----	-----0	-----	
10	-----0	-----	-----0	-----	-----0	-----	
11	-----0	-----	-----0	-----	-----0	-----	
12	-----0	-----	-----0	-----	-----0	-----	
14	-----0	-----	-----0	-----	-----0	-----	
15	-----0	-----	-----0	-----	-----0	-----	
16	-----	-----	-----	-----	-----	-----	
17	-----0	-----	-----0	-----	-----0	-----	
	-3-2	-----2-3-	-3-2	-----2-3-	-3-2	-----2-3-	
	< -SD+ >		< -SD+ >		< -SD+ >		
	SAMPLE 1		SAMPLE 2		SAMPLE 3		
	RESULT	%BIAS/MEDIAN	RESULT	%BIAS/MEDIAN	RESULT	%BIAS/MEDI	
1	21.00	-76.40	17.00	-84.82	22.00	-75.28	
2	94.00	5.62	115.00	2.68	99.00	11.24	
3	89.00	0.00	104.00	-7.14	81.00	-8.99	
4	107.00	20.22	107.00	-4.46	92.00	3.37	
5	86.00	-3.37	136.00	21.43	101.00	13.48	
6							
7							
8	92.00	3.37	114.00	1.79	89.00	0.00	
9	79.00	-11.24	89.00	-20.54	89.00	0.00	
10	95.00	6.74	112.00	0.00	82.00	-7.87	
11	75.00	-15.73	105.00	-6.25	74.00	-16.85	
12	86.00	-3.37	87.00	-22.32	97.00	8.99	
13							
14	121.00	35.96	123.00	9.82	119.00	33.71	
15	88.00	-1.12	118.00	5.36	85.00	-4.49	
16							
17	95.00	6.74	130.00	16.07	90.00	1.12	
18							
MEDIAN	89.000		112.00		89.00		
MEAN	89.64		109.45		89.00		
EST.SD	11.40		17.67		11.23		
%CV	12.72		16.14		12.62		

Figure 1. Monthly report

Table 2. Cumulative statistics .

**IAEA EXTERNAL QUALITY ASSESSMENT
SCHEME FOR THYROID RELATED HORMONES
SIX MONTHLY RUNNING CUMULATIVE STATISTICS
APRIL UPTO SEPTEMBER 1992
DISTRIBUTION 1 TO 6**

LAB	ANALYTE : MEAN BIAS (%)	T	VARBIAS	nMol/L	N
1	-6.02		5.16		17
2	1.47		5.96		17
3	17.87		109.82		7
4	24.24		34.14		17
5	0.82		11.78		17
6	0.62		3.32		7
7	-		-		-
8	-9.49		8.16		17
9	-14.49		9.82		17
10	-7.33		6.34		17
11	46.21		121.03		17
12	18.96		31.99		17
14	6.44		18.67		17
15	9.20		18.52		17
16	10.04		14.43		17
17	10.29		16.96		17

LAB	ANALYTE : MEAN BIAS (%)	T3	VARBIAS	nMol/L	N
1	8.44		30.58		17
2	11.90		24.51		17
3	4.01		56.77		17
4	66.45		73.24		17
5	-9.82		17.92		17
6	0.86		5.08		7
7	-19.95		17.88		17
8	1.14		11.04		17
9	-22.93		39.40		17
10	-3.21		11.41		17
11	-5.93		25.38		17
12	-6.67		24.99		17
14	13.60		28.40		17
15	2.32		15.56		17
16	39.86		33.73		17
17	5.86		40.11		17

LAB	ANALYTE : MEAN BIAS (%)	TSH	VARBIAS	mU/L	N
1	9.32		9.23		17
2	-17.25		18.89		17
3	11.42		26.89		17
4	35.55		140.99		17
5	4.59		10.03		17
6	-4.97		8.06		7
7	0.48		15.22		17
8	0.19		11.51		17
9	-5.17		23.32		7
10	2.81		18.42		17
11	-		-		-
12	-6.13		41.33		17
14	-		-		-
15	22.39		28.69		17
16	1.25		10.69		17
17	-41.42		16.01		17

Table 3. Cumulative statistics .

**IAEA EXTERNAL QUALITY ASSESSMENT
SCHEME FOR THYROID RELATED HORMONES
SIX MONTHLY RUNNING CUMULATIVE STATISTICS
OCTOBER UPTO MARCH 1993
DISTRIBUTION 7 TO 12**

LAB	ANALYTE : T4		nMol/L	N
	MEAN BIAS (%)	VARBIAS		
1	-19.88	28.01		17
2	5.03	4.85		17
3	-15.04	19.07		7
4	19.51	36.99		17
5	7.36	10.73		17
6	-3.79	4.63		2
7	-	-		-
8	-3.59	5.53		17
9	-11.92	17.72		8
10	-5.94	6.28		17
11	-5.72	12.32		17
12	0.84	10.68		17
14	18.27	14.59		8
15	-0.32	8.80		11
16	28.15	1.28		4
17	13.10	8.25		17

LAB	ANALYTE : T3		nMol/L	N
	MEAN BIAS (%)	VARBIAS		
1	7.88	17.29		17
2	-4.02	14.09		17
3	-8.11	11.69		17
4	48.29	79.08		17
5	-4.79	43.23		17
6	5.42	5.36		2
7	-6.50	13.93		17
8	-2.61	5.70		17
9	4.61	12.79		8
10	-2.81	11.89		17
11	-0.80	10.34		17
12	5.35	20.58		17
14	15.39	18.65		8
15	5.41	9.19		17
16	92.19	49.89		4
17	16.49	39.26		17

LAB	ANALYTE : TSH		mU/L	N
	MEAN BIAS (%)	VARBIAS		
1	4.99	12.57		16
2	-4.02	61.99		17
3	-5.70	12.12		17
4	7.60	57.54		17
5	-1.67	14.14		17
6	6.04	18.82		2
7	5.39	26.78		17
8	-2.33	6.38		17
9	1.23	6.08		8
10	9.69	30.13		17
11	-	-		-
12	0.40	64.11		17
14	-	-		-
15	16.69	18.07		17
16	-20.09	39.48		4
17	227.38	886.66		17

Between-laboratory variability

The mean between-laboratory coefficient variation (CV) of T4, T3 and TSH for the 34 distributed samples was 16.9%, 29.5% and 27.2% respectively with a range of the CV means for individual pools of 5.8-48.9% for T4, 12.3-76.3% for T3 and 12.7-94.6% for TSH (Table 4).

The interlaboratory imprecision profiles of T4, T3 and TSH are shown in Fig 2. As compared to last years scheme,⁽⁶⁾ TSH assays exhibited much

less imprecise results and T4 assays had somewhat better precision, but not so for T3 assays.

Reproducibility of the ALTM

The ALTM of T4, T3 and TSH assays for all pools were sufficiently reproducible. The mean CV of ALTM of T4 and T3 was highest for pool 7. The mean CV of ALTM of all pools for T4, T3 and TSH was 3.9%, 5.6% and 3.3% respectively (Table 5).

Table 4. Between-laboratory variability.

	T4	T3	TSH
Mean between-LAB CV (%)	16.9	29.5	27.2
Range	5.8-48.9	12.3-76.3	12.7-94.6

Table 5. Reproducibility of ALTLM

POOL	N	T4		T3		TSH	
		ALTM	%CV	ALTM	%CV	ALTM	%CV
1	5	96	1.7	1.8	4.6	1.6	3.1
2	5	113	2.4	2.0	5.2	1.7	3.7
3	5	93	3.4	1.6	4.9	1.9	3.9
4	5	90	0.7	3.3	6.8	1.9	3.3
5	5	92	4.1	1.9	3.9	2.2	4.8
6	5	90	3.8	2.0	5.1	13.1	2.8
7	2	19	11.7	0.7	10.3	0.3	3.2
8	2	17	3.7	0.6	3.8	9.4	1.3
MEAN			3.9		5.6		3.3

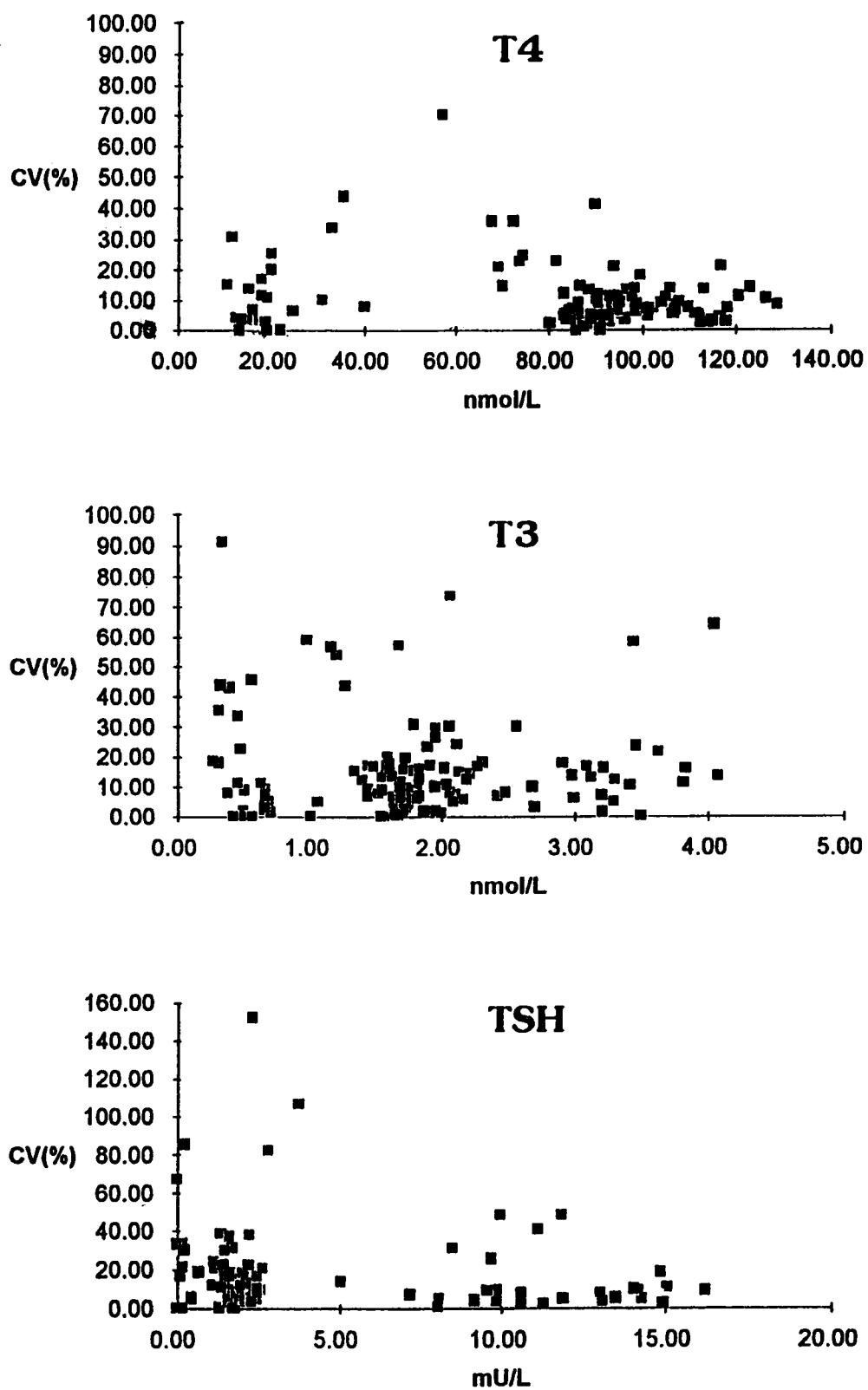


Figure 2. Interlaboratory imprecision profiles.

Recovery results

The recovery of T4, T3 and TSH at individual laboratories ranged from 0 to 144%, 59 to 159% and 69 to 126% respectively. The consensus recovery of T4 was only 85% which most likely resulted from technical errors in

measuring T4 concentrations during the preparation of the spiked T4 pool (pool 2). The consensus recovery for T3 was 106%. For TSH, the recovery results were 109% and 91% for pool 6 (human serum) and pool 8 (horse serum), respectively (Table 6).

Table 6. Recovery results.

T4		T3	
Base pool (pool 1) Euthyroid		Base pool (pool 3) : Euthyroid	
ALTM	96 mU/L	ALTM	1.6 mU/L
Recovery pool (pool 2)		Recovery pool (pool 4)	
T4 added	20 mU/L	T3 added	1.5 mU/L
ALTM	113 mU/L	ALTM	3.3 mU/L
Recovered T4	17 mU/L	Recovered T3	1.7 mU/L
Recovery	85%	Recovery	106%
TSH		TSH	
Base pool (pool 5) Euthyroid		Base pool (pool 7) : Euthyroid	
ALTM	2.2 mU/L	ALTM	0.3 mU/L
Recovery pool (pool 6)		Recovery pool (pool 8)	
TSH added	10 mU/L	TSH added	10.0 mU/L
ALTM	13.1 mU/L	ALTM	9.4 mU/L
Recovered TSH	10.9 mU/L	Recovered TSH	9.1 mU/L
Recovery	109%	Recovery	91%

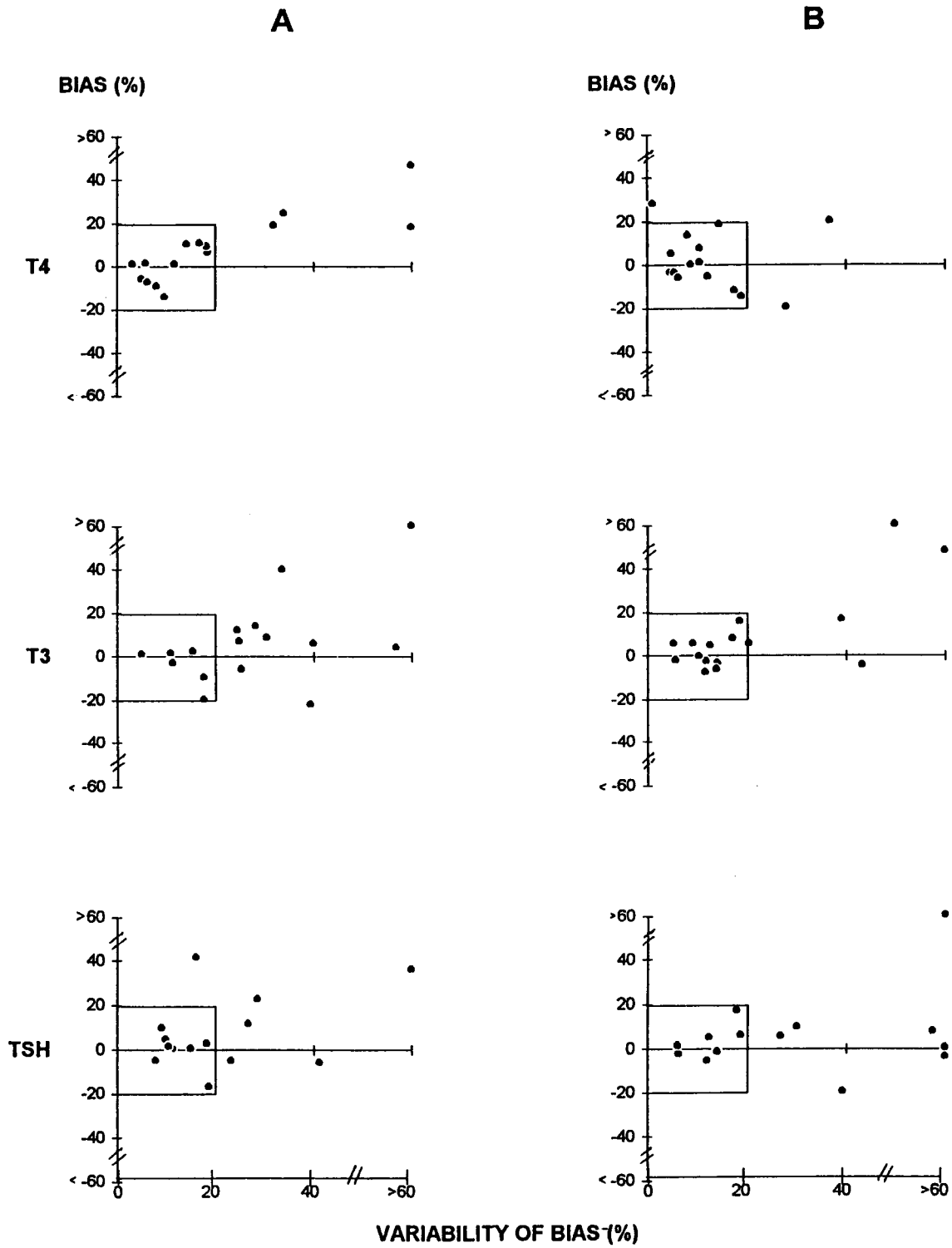


Figure 3. Cumulative bias vs variability of bias in six-month cycle

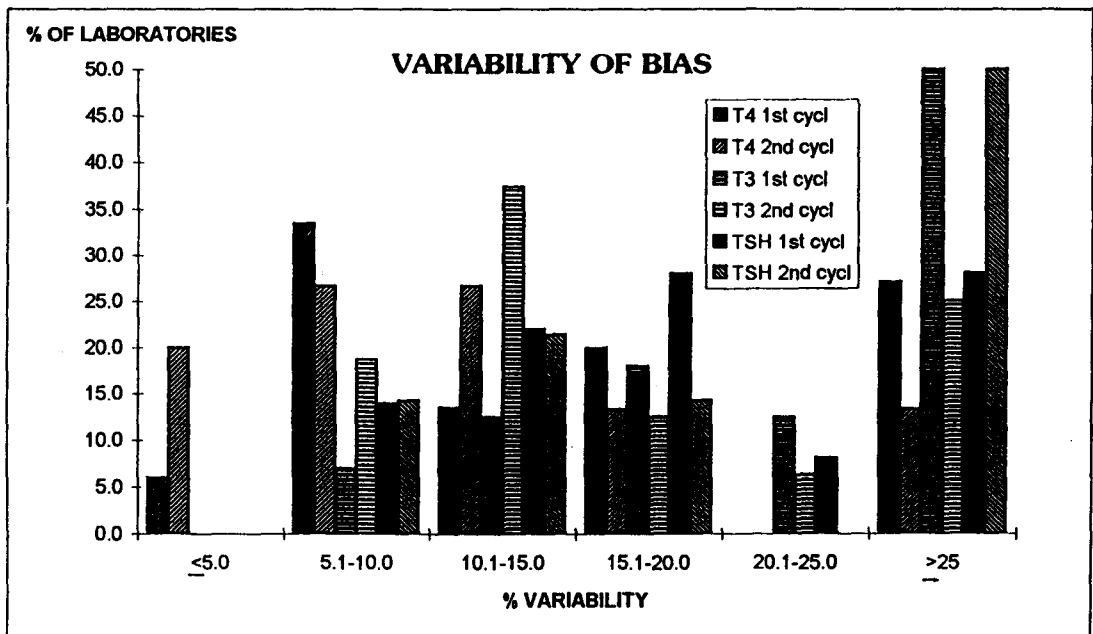
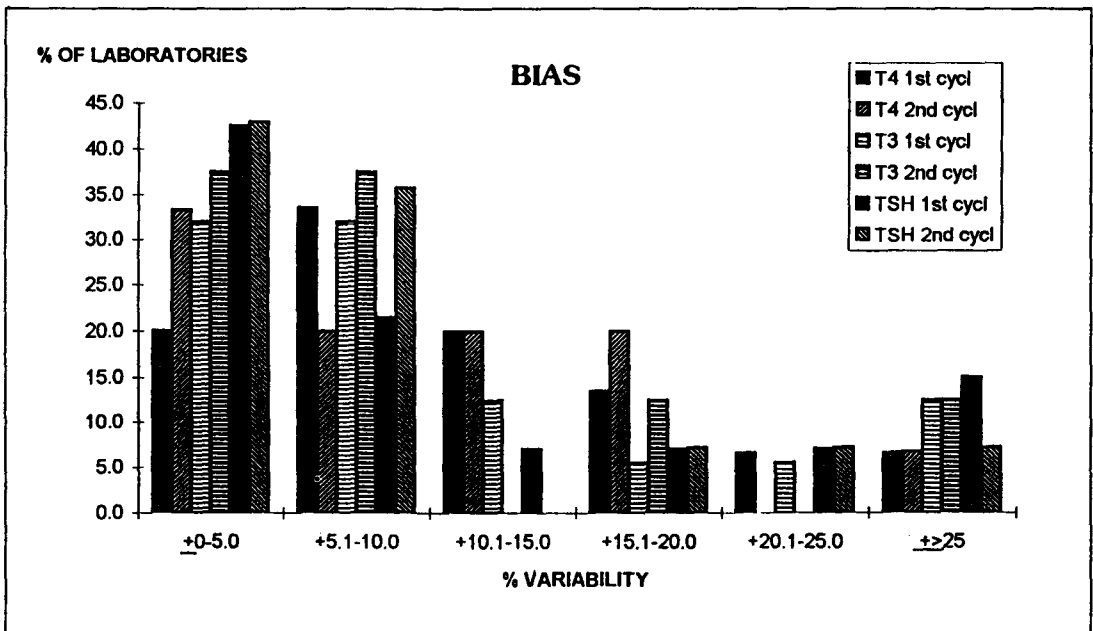


Figure 4. Distribution of laboratories according to bias and variability of bias of T4, T3 and TSH.

Assessment of bias

The six-month running cumulative bias and variability of bias were calculated from the median. The mean bias in individual laboratories for T4, T3 and TSH ranged from -20 to +46, -22 to +92 and -41 to +227, respectively (Tables 2 and 3). The median bias, ignoring sign, of T4, T3 and TSH for the first six-month cycle vs the second six-month cycle was 9.5 vs 7.4, 6.7 vs 5.4, and 6.1 vs 5.4, respectively, and the variability of bias was 14.4% vs 10.7%, 25% vs 13.9% and 18.9% vs 18.8% respectively.

Plots of cumulative bias against variability of bias are shown in Figure 3. Significant improvements in assay performance of T4 and T3 were noted in the second six-month cycle, but less so for TSH. The distribution of laboratories according to bias and variability of bias is shown in Figure 4. Improvement is obvious for the second six-month cycle for T4 and T3, but not so for TSH.

Outlier in each pool

Assay values outside $ALTM \pm 3 SD$ are considered as outliers. The mean percentage of outliers of T4, T3 and TSH in each pool ranged from 0-5%, 0-7.5% and 0-8.8% respectively and the mean of outliers of all pools was 3.1%, 3.8% and 3.9% for T4, T3 and TSH, respectively. Figure 5 demonstrates the results of assay performance from all laboratories for the whole year for T4, T3 and TSH assays of base pools and manipulated pools.

Eight participants exhibited satisfactory

assay performance, 4 laboratories occasionally failed to meet acceptable limits and 3 laboratories frequently showed unacceptable performance. One of these exhibited poor performance on TSH assays only. One laboratory provided very poor participation, hence the assay performance can not be assessed.

Discussion

The results of data analysis from Bangkok Center may not be robust because of the small number of participating laboratories which resulted from the drop out of 7 laboratories from two countries as already mentioned. However, during the IAEA Meeting of National Coordinators at Colombo, Sri Lanka over 12-16 July 1993 for presenting the results of 9 months data collection by the three organizing centers, we found that our ALTM was similar to Seoul Center where there were 50 participating laboratories, and our result was not much different from Lahore Center results.⁽⁶⁾

At the IAEA Meeting of National Coordinators, Colombo, Sri Lanka, 14-18 October 1991.⁽⁶⁾ We reported significant improvements in performance of radioimmunoassays for T4, T3 and TSH in the second year scheme as compared to the first year.

When comparing the present third year EQAS, to the previous year scheme,⁽⁶⁾ there is also considerable improvement in performance of TSH assays since 71% of the laboratories achieved a bias

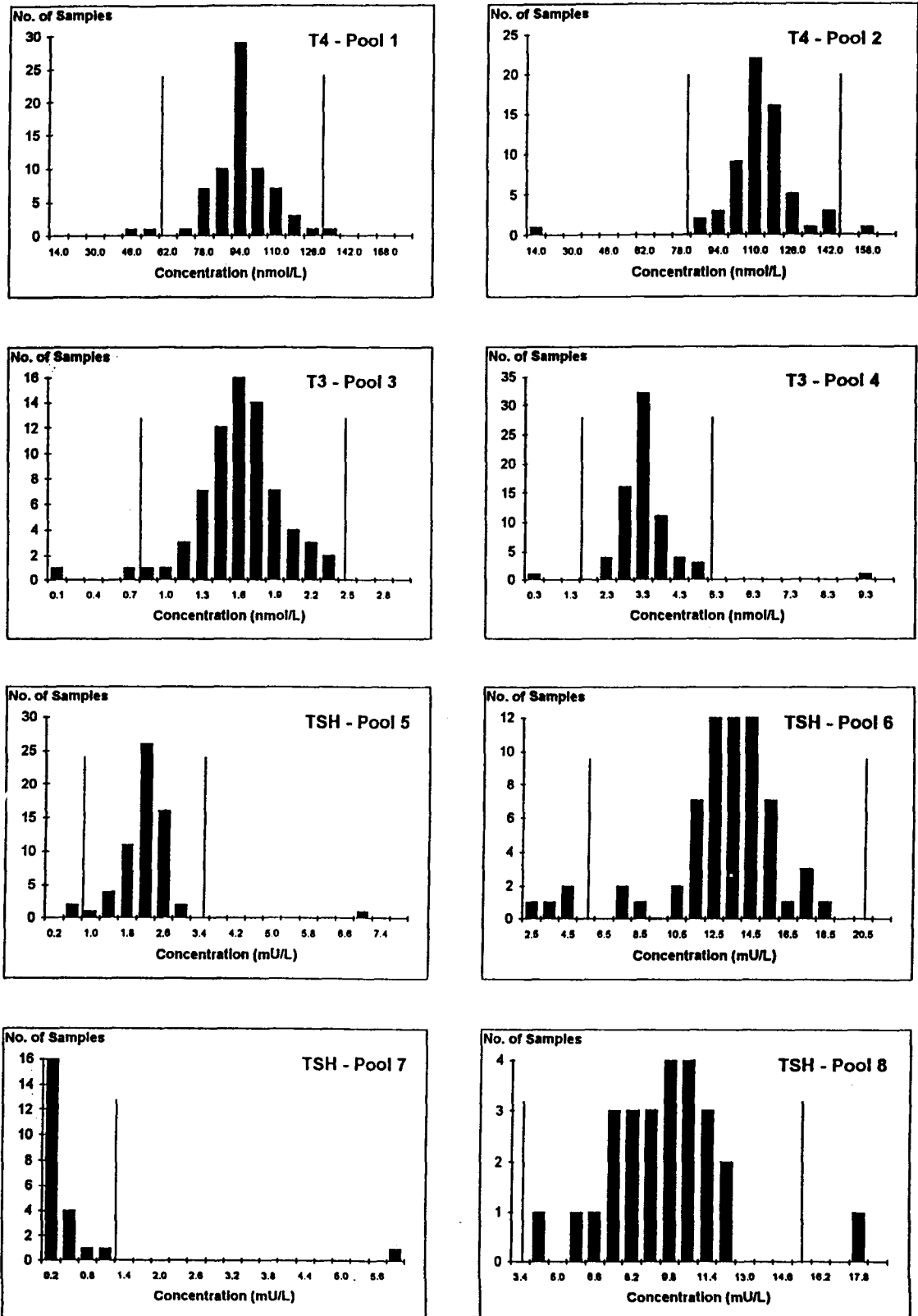


Figure 5. Histogram of individual data for T4, T3 and TSH of base pool and manipulated pools. Two vertical lines represent $ALTM \pm 3SD$.

below $\pm 10\%$ as compared to 46% in the previous scheme, and the number of laboratories with variability of bias below 25% increased from 27% in the previous scheme to 60% in the present scheme. However, one participating laboratory persistently exhibited poor performance in TSH assays only, which indicates a problem possibly from the reagent used for the assay.

For T4 assays, only 56% of the laboratories achieved a bias less than $\pm 10\%$ which is lower than in the previous scheme (68%), but the number of laboratories with variability of bias below 10% was increased from 16% over the previous scheme⁽⁶⁾ to 43% in this scheme. The present scheme used 2 pools of horse sera where the levels of T4 were very low, hence the actual performance of the present scheme should be better than in the previous one.

The performance of T3 assays was also improved since 84% of the laboratories achieved a bias below $\pm 20\%$ as compared to 78% in the previous scheme,⁽⁶⁾ and the number of laboratories with variability of bias below 20% increased from 38% to 53%.⁽⁶⁾

Conclusion

The results of present EQAS indicate further improvement in radioimmunoassay performance in most participating laboratories. Three laboratories frequently showed poor performance and only one laboratory had very poor return results. The improvement was obvious for TSH, but less so for T3 and T4 assays. The scheme was well

accepted by the participants and created interest and concern in quality control practices as shown by the continuous improvement of laboratory performance.

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