MODIFICATION OF THYROID STIMULATING HORMONE ESTIMATION METHOD IN ALPHA PRIME LS SYSTEM (SFRI)

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ABSTRACT

The alpha prime LS (APLS) system consists of both enzyme linked immunosorbant assay (ELISA) and chemiluminescence immunoassay (CLIA) processing arrangements. All the programmes are open and accessible to modification. But, as CLIA kits are system dedicated kits, so the option for user defined programme may only be utilized for modification as and when it is absolutely necessary. The author had some problem during routine performances with thyroid stimulating hormone (TSH) Monobind, U.S.A., kits. So, the author modified the incubation temperature, time of decontamination, use of signal reagents and finally the calibration pattern.

KEY WORDS

CLIA, ELISA, RIQAS, SDEC, SRC, APLS, RLU.

INTRODUCTION

External and internal quality assessment is a routine procedure in any standard laboratory. In the Peerless Hospital laboratory Biorad controls, lyphocheck immunoassay level 1,2,3 (L1, L2, L3) are used for IQA and EQA. But after rigorously following the Monobind U.S.A., product insert for TSH estimation the values did not match. The surprising observation was that there was no consistent bias pattern of such mismatching. Moreover, on an average, out of 20 samples 6-7 samples were found out to be showing 0 μ iu/ml TSH concentration. Both the observations are sufficient enough to feel that the method needs thorough modification. The second alternative could have been to change the whole system as all CLIA kits are system dedicated but seemed to the author as an impractical approach as the instrument used is an expensive one and newly installed. Regarding decontamination steps, which are very important in CLIA, specifically no guidelines were given in the insert. Hence, the author had to modify the methods as well as to determine optimum decontamination time. As no proper user defined programme sheet was available the user had to prepare it herself for further use.

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MATERIALS AND METHODS

1. TSH kit from Monobind, U.S.A.
2. Methanol
3. Solution for decontamination (SDEC), 1 ml /litre deionised water, solution rinsing cuvette (SRC), 500 μ l/litre and wash solution, 20 ml/ litre deionised water.
4. Precicontrol U1 and U2 from Roche Diagnostics, Randox international quality assessment sample (RIQAS), BioRad lyophilized immunoassay L1, L2, L3 controls.
5. TSH cal set 1 & 2, Roche Diagnostics.

Samples, controls were poured in sample cups which were placed in APLS rack in respective positions. Wells as per requirement were kept in CLIA plate in the workstation. Incubation temperature can be chosen from 25° to 45° Celsius (1). Here, the reaction temperature was kept at 37° Celsius. Signal reagents A and B were collected from the 7 ml containers in two separate big, clean, sterile dark colored containers labeled as signal A & B. The signal reagents were mixed in equal ratio, 100 μ l per test plus dead volume. Every new kit was calibrated immediately after opening. Chosen standards were placed in a minikit in the respective positions. Standards chosen were, position 1- TSH cal set 1 (0 μ iu/ml), position 2- TSH cal set 2 (1.5 μ iu/ml), positions 3, 4, 5, 6 were 5, 10, 20, 40 μ iu/ml Monobind standards respectively. Incubation time was 45 minutes.

Precinorm U1 and U2, BioRad L1, L2, L3, RIQAS samples were reconstituted as per instructions. Aliquots were kept at -20° Celsius and thawed only once before use. RIQAS samples retain their activity for 30 days and Bio Rad controls for 20 days at -20° Celsius (2,3).

The user defined programme sheet was prepared by the author herself. Here is the comparison of the two methods i.e. Monobind U.S.A., product insert method and the modified method.

RESULTS AND DISCUSSION

Four important changes were made from the original product insert.

1. The incubation temperature was maintained at 37° Celsius instead of room temperature. In this context it

can be said that instead of room temperature a specific temperature needs to be mentioned in the product insert as it varies with climate. Table 1 is self explanatory why the incubation temperature was chosen at 37° Celsius. Five human samples, at random, were chosen and TSH were estimated (Table 1)(4).

2. The relative light units (RLUs) of 0, 0.5, 2.5, 5.0, 10, 20, 40 μ iu/ml (Monobind, U.S.A.) standards were measured using 5 pairs of signal A and B reagents from one 960 determination kit instead of mixing all signal A in one and all signal B in another container. Signal reagents were prepared freshly. Table 2 and Figure 1 point out some vital things :

- The whole signal A & B should be kept in two containers as basal RLU varies from one vial to another (Figure 1). The graph shows greater

User Defined Programme for TSH Estimation

		MONOBIND, U.S.A	MODIFIED METHOD
1.	Sample volume (μ l)	50	50
2.	Reagent volume (μ l)	100	100
3.	Decontamination time (seconds)	not suggested	3
4.	Number of de – contamination steps	not suggested	3
5.	Incubation time (minutes)	45	45
6.	Incubation temperature	room temperature	37°C
7.	Number of washes after incubation	3	3
8.	Washing medium	wash buffer (20ml wash concentrate in 980ml deionized water)	wash buffer (20ml wash concentrate in 980ml deionized water)
9.	Signal reagent (μ l)	100	100
10.	Signal reagent incubation time (minutes)	5	5
11.	Signal reagent incubation temperature	room temperature	37°C
12.	Readings taken	relative light units; in luminometer; 0.2 seconds/well	relative light units; in luminometer; 0.2 seconds/well
13.	Calibrators chosen (μ iu/ml)	0,0.5,2.5,5.0,10,20,40 (Monobind, U.S.A)	0 (Roche),1.5(Roche), 5.0,10,20,40 (Monobind, U.S.A)

Decontamination steps: (1) after putting 50 μ l sample in the well; (2) after putting conjugate and mixing both the sample and conjugate and (3) after pouring signal reagent.

Table 1 : COMPARATIVE STUDY OF ELECSYS 2010 AND ALPHA PRIME AT THREE DIFFERENT TEMPERATURES

Sl. No.	Sample ($\mu\text{iu/ml}$)	Elecsys 2010 25°C	APLS		
			25°C	30°C	37°C
1	Human Sample, 1	3.9	4.8	5.9	3.85
2	Human Sample, 2	0.7	1.5	1.6	0.61
3	Human Sample, 3	6.7	8.0	8.2	6.6
4	Human Sample, 4	25.9	21	20.9	26.8
5	Human Sample, 5	13.8	8.2	10.1	14.8
6	(Precicontrol U1, 1.31)	1.3	2.2	2.5	1.26
7	(Precicontrol U2, 7.14)	7.1	9.0	10.5	7.0
8	RIQAS CONTROL				
	i) 14	13.6	10.9	16.6	13.5
	ii) 0.164	0.2	0.2	0.5	0.18
	iii) 1.426	1.45	1.6	1.6	1.5
9	BIORAD CONTROLS				
	i) Level 1; 0.47	0.5	1.0	1.2	0.5
	ii) Level2; 6.7	6.8	5.2	7.0	6.8
	iii) Level 3; 34.8	35.1	19.6	22.8	35.2

- chance of erroneous reports.
 - The overlapping values of 0 $\mu\text{iu/ml}$ and 0.5 $\mu\text{iu/ml}$ concentrations enhance the risk factor of wrong reporting of low values and inability of detecting hyperthyroid subjects.
 - The author's finding does not suggest the Indian distributor / Monobind, U.S.A. to send signal A/ B in one container each without prior intimation to the customer or without mentioning about the change in the label on the kit or product insert.
3. Decontamination time has not been specified in the product insert. So, the author herself had to set the optimum time for decontamination. The optimum time was found out to be 3 seconds (Table 3).
 4. The 0 $\mu\text{iu/ml}$ TSH concentration of Monobind kit has always shown very high RLU (Table 2). The values of 0 $\mu\text{iu/ml}$ and 0.5 $\mu\text{iu/ml}$ were also overlapping. TSH cal set 1 (0 $\mu\text{iu/ml}$) and cal set 2 (1.5 $\mu\text{iu/ml}$), instead of, Monobind standards 0 and 2.5 $\mu\text{iu/ml}$ were used and better results were found out (Table 4).

Figure 1 **Table 2**

Standard Concentrations ($\mu\text{iu/ml}$)	RLU Signal reagents				
	pair1	pair2	pair3	pair4	pair5
0	718.2	1193	872	1392	989
0.5	611.3	1190	1013	1124	911
2.5	2192	3250	5732	6892	4593
5.0	3168	5359	10264	12261	9789
10.0	6810	9626	14230	15179	15112
20.0	14593	16215	22028	23129	25179
40.0	26092	29543	36379	30149	37373

Table 3

BIORAD CONTROL (μ iu/ml)	Time of decontamination (seconds)		
	1	2	3
0.47 (L1) (0.27 - 0.67)	0.25 $\pm$ 0.11	0.6 $\pm$ 0.24	0.5 $\pm$ 0.15
6.7 (L2) (5.3 – 8.1)	5.8 $\pm$ 1.35	9.0 $\pm$ 1.49	6.5 $\pm$ 0.45
34.8 (L3) (26.8 – 42.8)	24.4 $\pm$ 2.8	26.2 $\pm$ 3.5	36.1 $\pm$ 4.2

Results given in the table are 60 days summary report.

Table 4 : Variability in RLU of the standards checked during calibrations

Concentrations (μ iu/ml)	1st Calibration RLU	2nd Calibration RLU	3rd Calibration RLU
0 (calset TSH)	626.7	611.0	619.2
1.5 (calset TSH)	865.4	820.2	871.8
5.0 (Monobind USA)	3765	3665	3750
10.0 (Monobind USA)	6842	6877	6901
20.0 (Monobind USA)	14693	14598	14704
40.0 (Monobind USA)	27091	26058	26111

Finally, at 37°Celsius with 45 minutes incubation time, using cal set 1, cal set 2, 5,10, 20, 40 μ iu/ml Monobind standards for calibration, given decontamination time 3 seconds 20 human samples were tested for TSH in APLS and Elecsys 2010 (Table 5).

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2. Lilac Medicare, Kolkata.
3. Roche Diagnostics (India) Ltd.
4. Peerless Hospital & B.K.Roy Research Centre, Kolkata.

Table 5 :Comparison of TSH values between Elecsys 2010 and APLS

Sl. No.	TSH (μ iu/ml)	
	APLS	Elecsys
1	5.5	5.4
2	2.6	2.6
3	5.7	5.5
4	4.4	4.6
5	21.5	21.8
6	0.5	0.55
7	26.4	26.3
8	3.9	3.9
9	0.15	0.15
10	6.8	6.9
11	14.1	14.3
12	1.31	1.38
13	1.9	1.9
14	0.6	0.7
15	9.8	9.6
16	40.8	39.8
17	36.6	37.0
18	8.9	9.0
19	4.9	4.85
20	31.6	31.2

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