APPENDIX

I. Reagent preparation.

[1.] Reagents for preparation of enzyme concentrates.

(1.) 0.01 mol/L Tris-HCl buffer, pH 7.7 for ALP

- Dissolved 1.2114 g of Tris (Hydroxymethyl) – aminomethane in 900 mL of distilled water and mixed until well dissolved.
- Adjusted pH to 7.7 with 1 N NaOH or 1 N HCl.
- Filled up with distilled water to 1,000 mL.
- Stored at 4°C.

(2.) 0.05 mol/L Tris-HCl buffer, pH 7.0 containing 10 mmol/L 2-mercaptoethanol and 10 mol/L EDTA for ALT

- Dissolved 1.8171 g of Tris (Hydroxymethyl) – aminomethane, 3.7224 g of EDTA and 0.699 mL 2-mercaptoethanol in 900 mL of distilled water and mixed until well dissolved.
- Adjusted pH to 7.0 with 1 N NaOH or 1 N HCl.
- Filled up with distilled water to 1,000 mL.
- Stored at 4°C.

(3.) 0.015 mol/L Tris-HCl buffer, pH 7.25 containing 10 mmol/L 2-mercaptoethanol and 2 mol/L EDTA for ALT

- Dissolved 1.8171 g of Tris (Hydroxymethyl) – aminomethane, 0.7445 g of EDTA and 0.699 mL 2-mercaptoethanol in 900 mL of distilled water and mixed until well dissolved.
- Adjusted pH to 7.25 with 1 N NaOH or 1 N HCl.
- Filled up with distilled water to 1,000 mL.
- Stored at 4°C.

(4.) 0.015 mol/L Tris-HCl buffer, pH 7.0 for AST

- Dissolved 1.8171 g of Tris (Hydroxymethyl) – aminomethane in 900 mL of distilled water and mixed until well dissolved.
- Adjusted pH to 7.0 with 1 N NaOH or 1 N HCl.
- Filled up with distilled water to 1,000 mL.
- Stored at 4°C.

(5.) 20 mmol/L Tris-HCl buffer, pH 7.4 containing 1 mol/L EDTA and 2 mmol/L dithiothreitol for LDH

- Dissolved 2.4228 g of Tris (Hydroxymethyl) – aminomethane, 0.372 g of EDTA and 0.3085 g of dithiothreitol in 900 mL of distilled water and mixed until well dissolved.
- Adjusted pH to 7.4 with 1 N NaOH or 1 N HCl.
- Filled up with distilled water to 1,000 mL.
- Stored at 4°C.

(6.) 20 mmol/L Tris-HCl buffer, pH 7.4 containing 1 mol/L EDTA, 2 mmol/L dithiothreitol and 50 mmol/L NaCl for LDH

- Dissolved 2.4228 g of Tris (Hydroxymethyl) – aminomethane, 0.372 g of EDTA, 0.3085 g of dithiothreitol and 2.925 g NaCl in 900 mL of distilled water and mixed until well dissolved.
- Adjusted pH to 7.4 with 1 N NaOH or 1 N HCl.
- Filled up with distilled water to 1,000 mL.
- Stored at 4°C.

(7.) 20 mmol/L Tris-HCl buffer, pH 7.4 containing 1 mol/L EDTA, 2 mmol/L dithiothreitol and 100 mmol/L NaCl for LDH

- Dissolved 2.4228 g of Tris (Hydroxymethyl) – aminomethane, 0.372 g of EDTA, 0.3085 g of dithiothreitol and 5.85 g NaCl in 900 mL of distilled water and mixed until well dissolved.
- Adjusted pH to 7.4 with 1 N NaOH or 1 N HCl.

- Filled up with distilled water to 1,000 mL.
- Stored at 4°C.

(8.) 20 mmol/L Tris-HCl buffer, pH 7.4 containing 1 mol/L EDTA, 2 mmol/L dithiothreitol and 140 mmol/L NaCl for LDH

- Dissolved 2.4228 g of Tris (Hydroxymethyl) – aminomethane, 0.372 g of EDTA, 0.3085 g of dithiothreitol and 8.19 g NaCl in 900 mL of distilled water and mixed until well dissolved.
- Adjusted pH to 7.4 with 1 N NaOH or 1 N HCl.
- Filled up with distilled water to 1,000 mL.
- Stored at 4°C.

[2.] Reagents for preparation of cholesterol concentrates

(1.) 20 g/L Heparin

- Dissolved 2 g of Heparin Lithium Salt in 100 mL of distilled water and mixed until well dissolved.
- Stored at room temperature.

(2.) 2.7 g/L $CaCl_2$

- Dissolved 2.7 g of $CaCl_2$ in 1,000 mL of distilled water and mixed until well dissolved.
- Stored at room temperature.

[3.] Reagents for SDS-PAGE

(1.) 1 M Tris-HCl buffer, pH 8.8

- Dissolved 121.14 g of Tris (Hydroxymethyl) – aminomethane in 900 mL of distilled water and mixed until well dissolved.
- Adjusted pH to 8.8 with 1 N NaOH or 1 N HCl.
- Filled up with distilled water to 1,000 mL.
- Stored at 4°C.

(2.) 1 M Tris-HCl buffer, pH 6.8

- Dissolved 121.14 g of Tris (Hydroxymethyl) – aminomethane in 900 mL of distilled water and mixed until well dissolved.
- Adjusted pH to 6.8 with 1 N NaOH or 1 N HCl.
- Filled up with distilled water to 1,000 mL.
- Stored at 4°C.

(3.) 20% SDS

- Dissolved 10 g of SDS in 40 mL of distilled water and mixed until well dissolved.
- Filled up with distilled water to 50 mL.
- Stored at room temperature.

(4.) 40% Acrylamide

- Dissolved 37.5 g of acrylamide and 1 g of bis-acrylamide in 90 ml of distilled water and mixed until well dissolved.
- Filled up with distilled water to 100 mL.
- Stored at 4°C.

(5.) 10% Ammonium persulfate

- Dissolved 10 g of ammonium persulfate in 90 ml of distilled water and mixed until well dissolved.
- Filled up with distilled water to 100 mL.
- Stored at 4°C.

(6.) 0.5% Bromophenol blue

- Dissolved 0.05 g of bromophenol blue in 10 mL of distilled water and mixed until well dissolved.

(7.) 0.5M EDTA

- Dissolved 1.8612 g of EDTA in 10 mL of distilled water and mixed until well dissolved.

(8.) 10X electrophoresis buffer

- Dissolved 15 g of Tris (Hydroxymethyl) – aminomethane, 72.5 g of glycine and 5 g of SDS in 400 mL of distilled water and mixed until well dissolved.
- Filled up with distilled water to 500 mL.
- Stored at 4°C.

(9.) Brilliant blue R-250 (staining solution)

- Dissolved 0.25 g of Brilliant blue R-250 in 400 mL of methanol and 70 mL of glacial acetic acid.
- Filled up with distilled water to 1,000 mL.
- Stored at room temperature.

(10.) Destaining solution (I)

- Mixed 400 mL of ethanol, 100 mL of acetic acid in 500 mL of distilled water.
- Stored at room temperature.

(11.) Destaining solution (II)

- Mixed 50 mL of methanol, 75 mL of acetic acid in distilled water.
- Filled up with distilled water to 1,000 mL.
- Stored at room temperature.

(12.) 6X sample buffer

- Mixed 6 mL of 100% glycerol, 3 mL of 1 M Tris-HCl buffer, pH 6.8, 240 µL of 0.5 M EDTA, 1.2 g of SDS, 600 µL of 2-mercaptoethanol and 0.2 mL of 0.5% bromophenol blue until well dissolved.

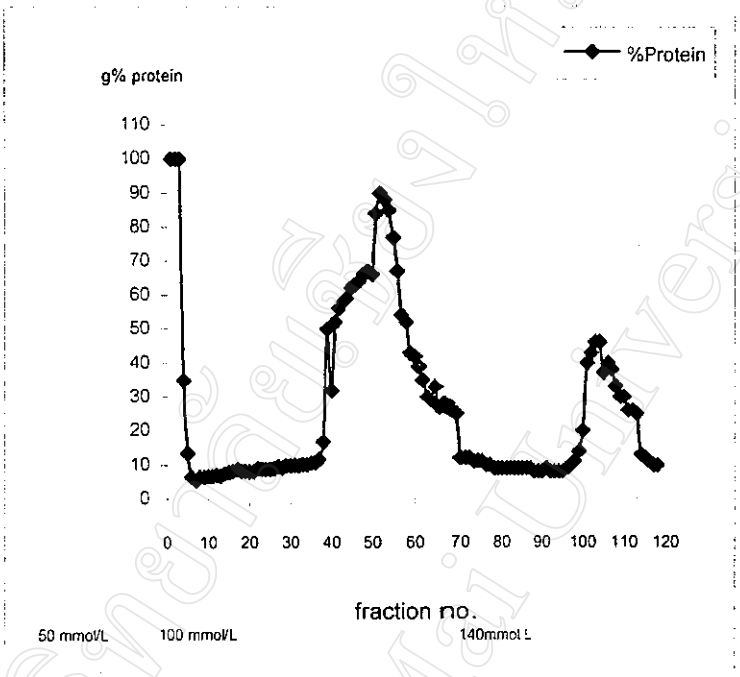
(13.) 7% separating gel

- Mixed 7.5 mL of 1 M Tris-HCl buffer, pH 8.8, 0.1 mL of 20% SDS, 3.5 mL of 40% acrylamide, 8.9 mL of distilled water, 0.2 mL of 10% ammonium persulfate.
- Added TEMED 30 μL in mixture.

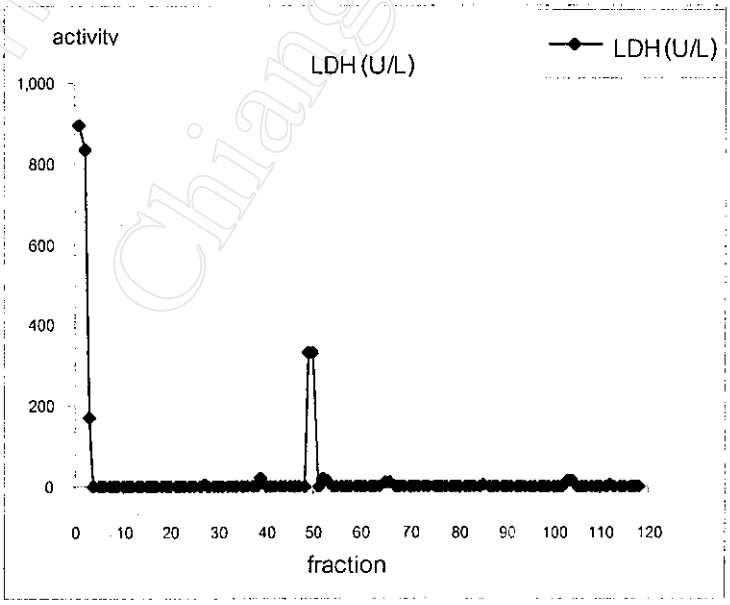
(14.) 3.75% stacking gel

- Mixed 0.75 mL of 1 M Tris-HCl buffer, pH 6.8, 30 μL of 20% SDS, 560 μL of 40% acrylamide, 4.7 mL of distilled water, 60 μL of 10% ammonium persulfate.
- Added TEMED 15 μL in mixture.

II. Profile of LDH eluted from DEAE-Sephacel ion exchange chromatography.



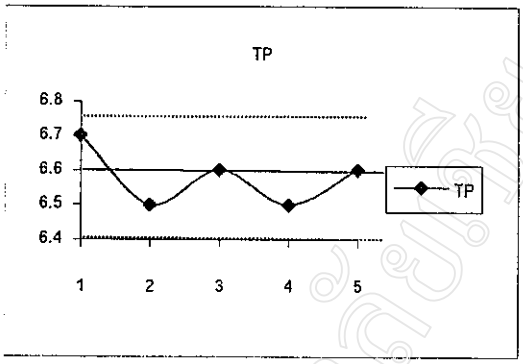
(1.) g% protein of each eluated fraction.

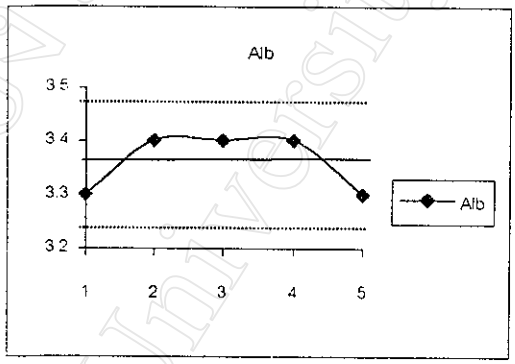


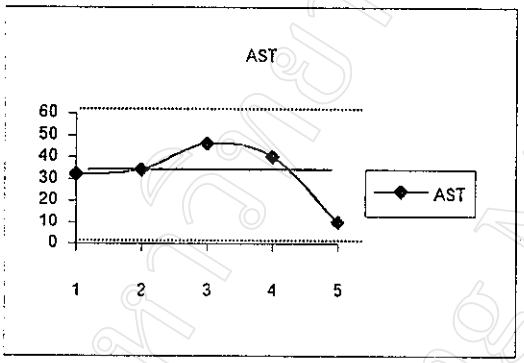
(2.) LDH activity of each eluated fraction.

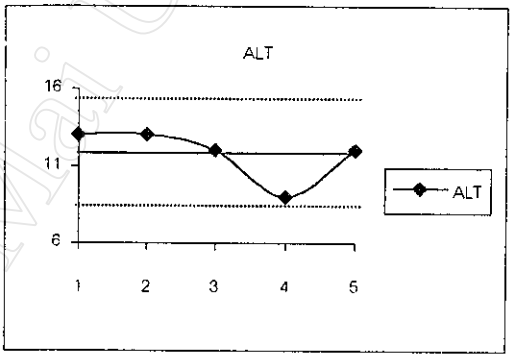
III. The quality control chart of protein and lipid constituents.

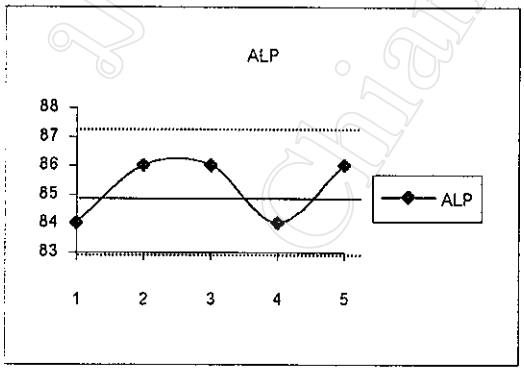
[1.] Distribution of protein, enzyme and lipid component concentration (or activities) in level I bovine matrix control serum containing 8.5% saccharose; Optimal condition variance (OCV).

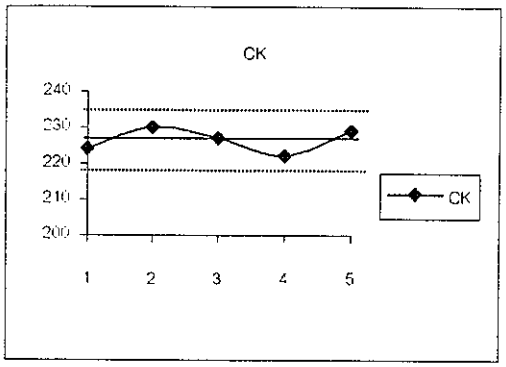


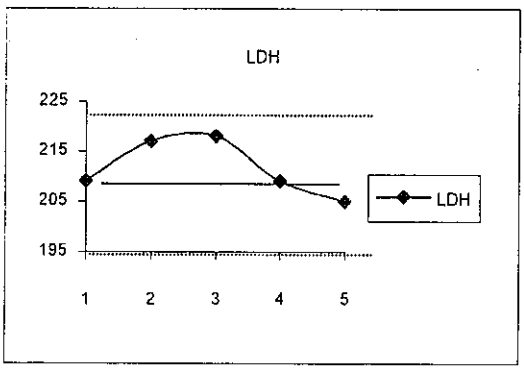


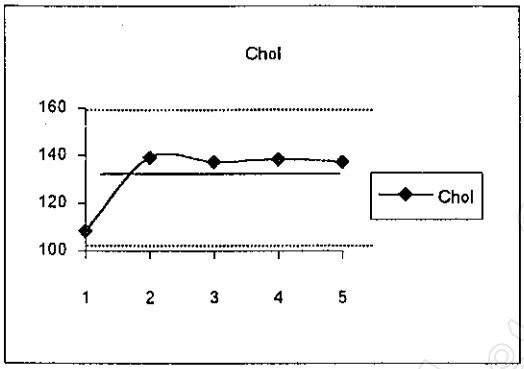


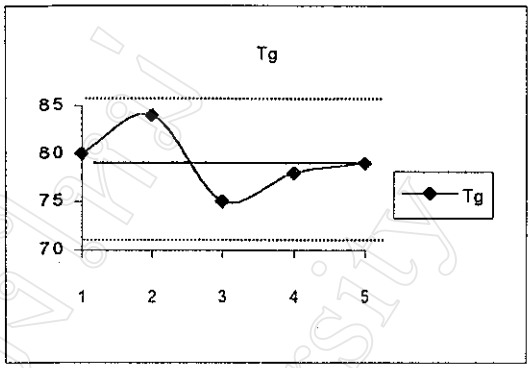


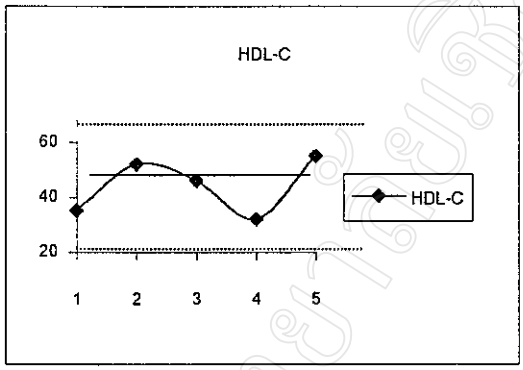


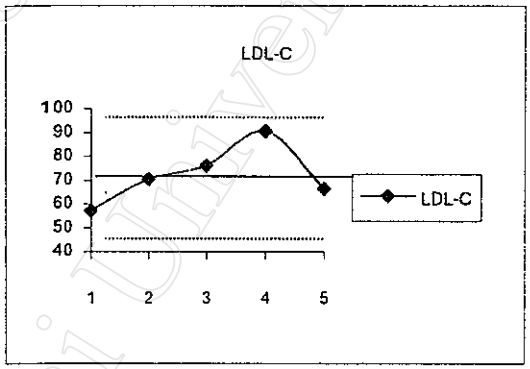












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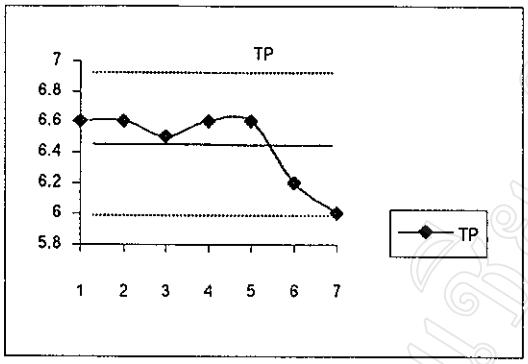

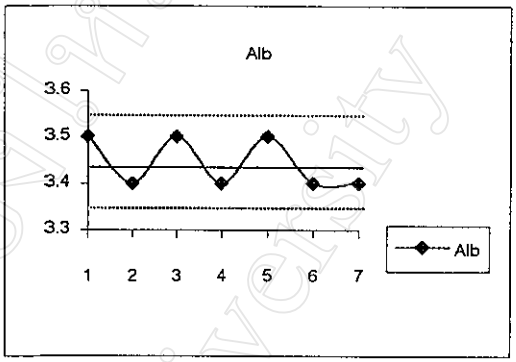

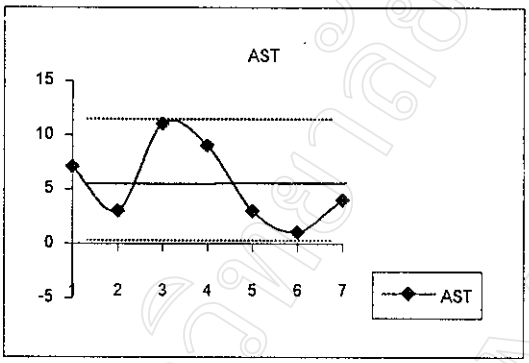

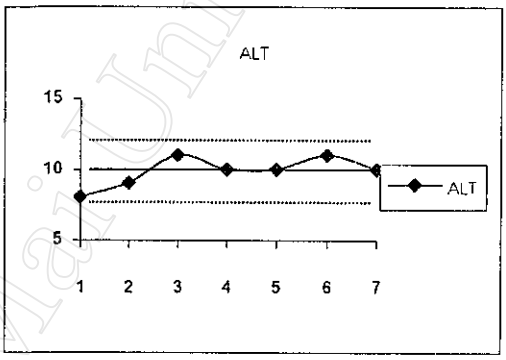

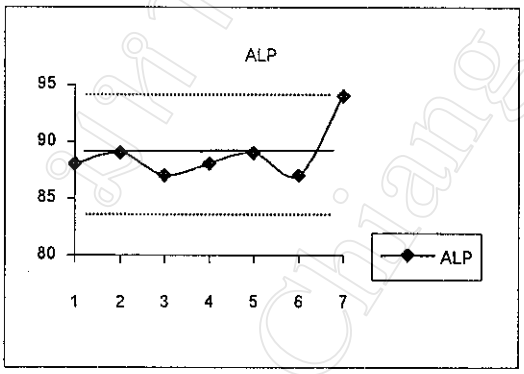

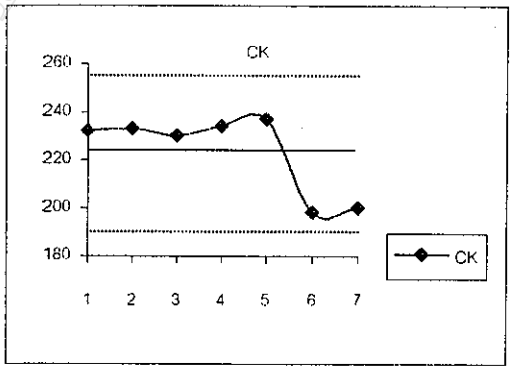

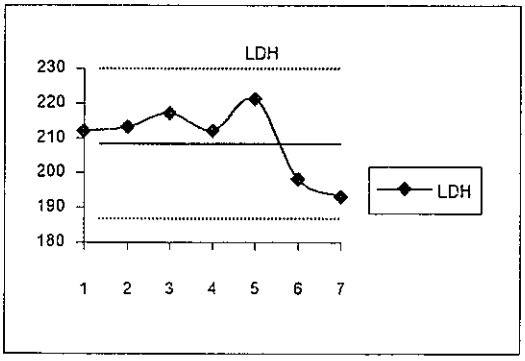

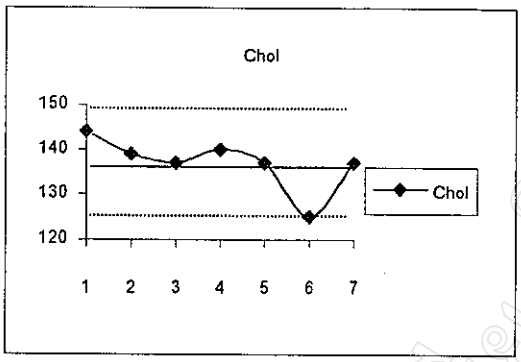

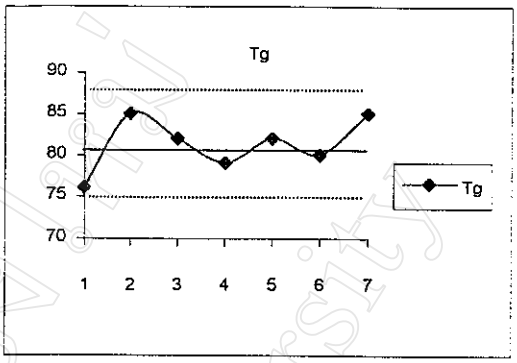

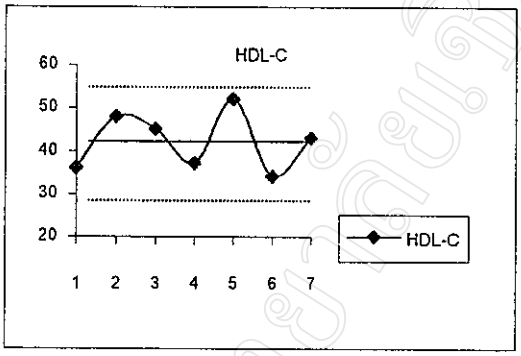

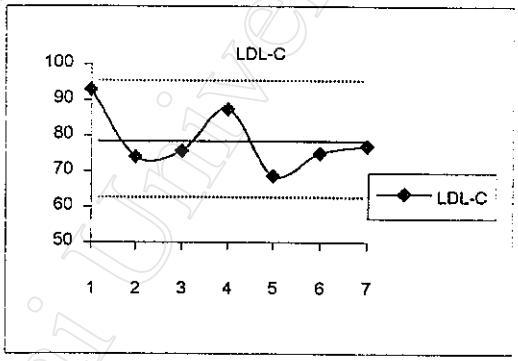

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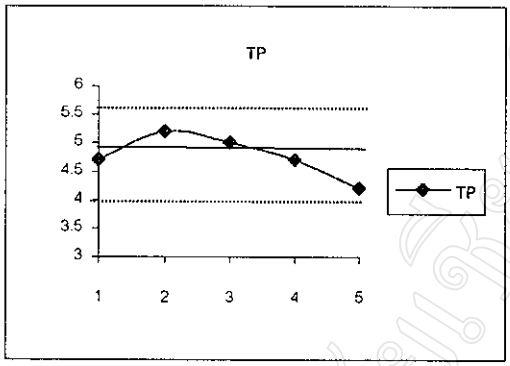

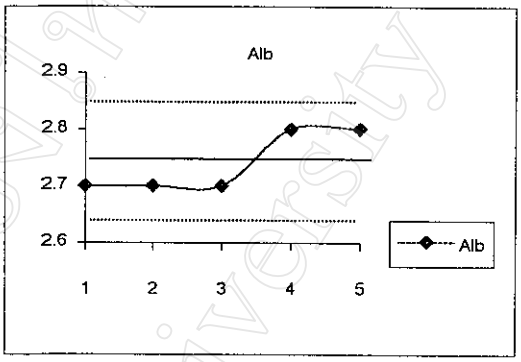

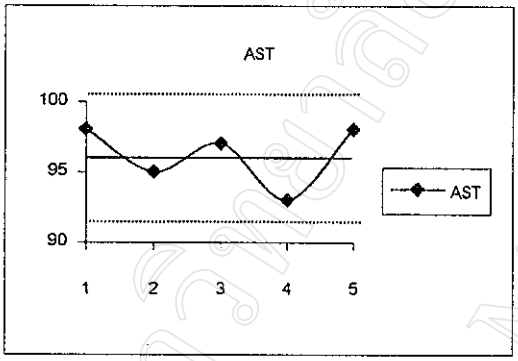

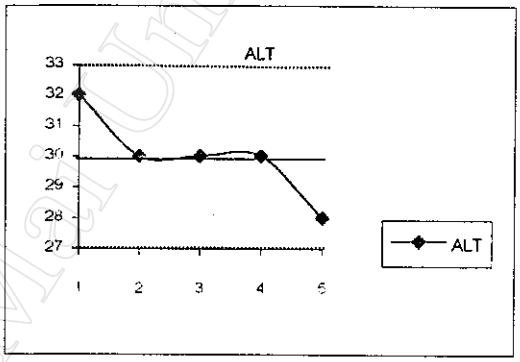

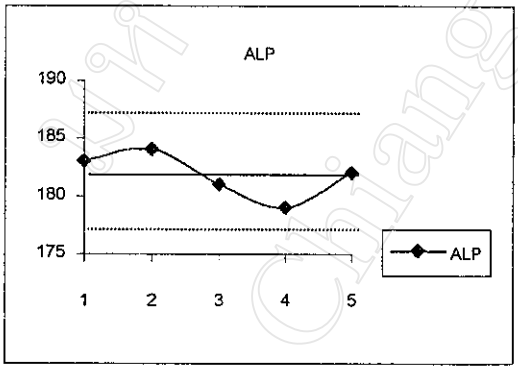

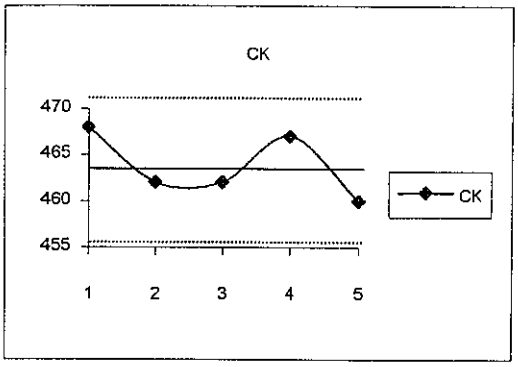

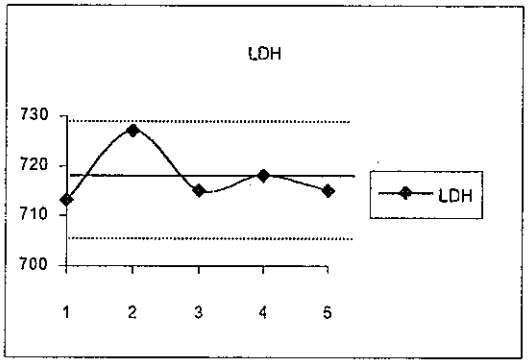

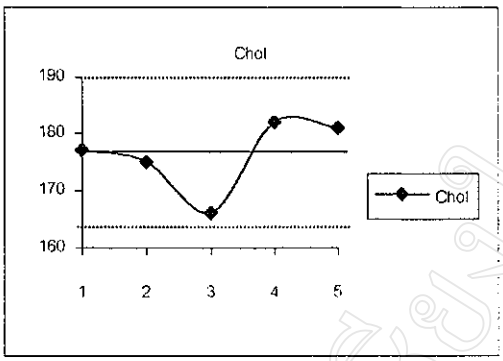

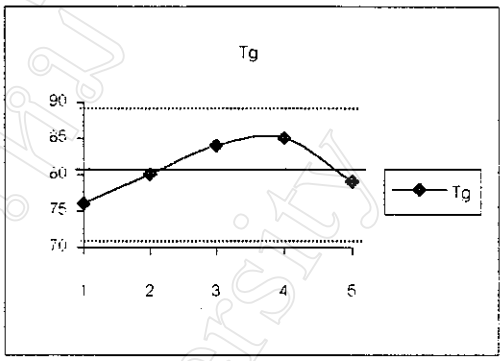

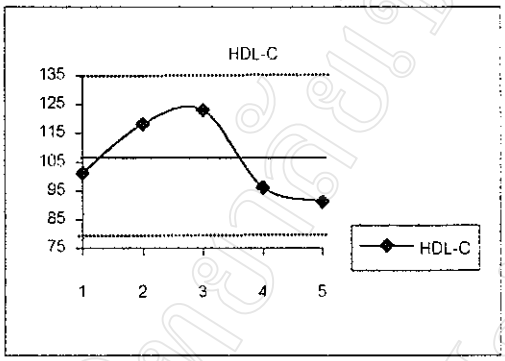

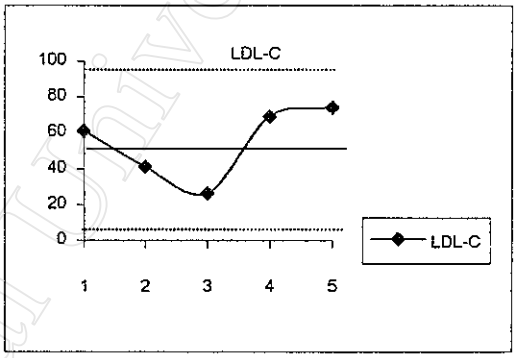

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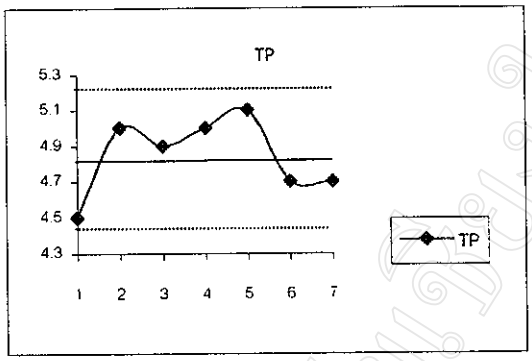
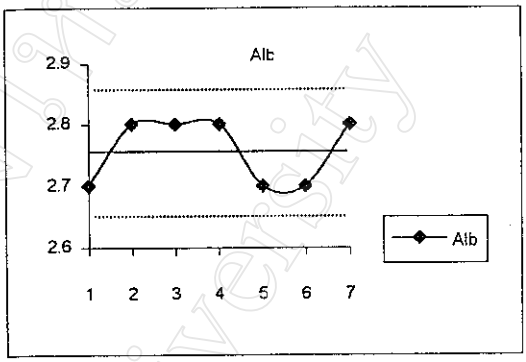
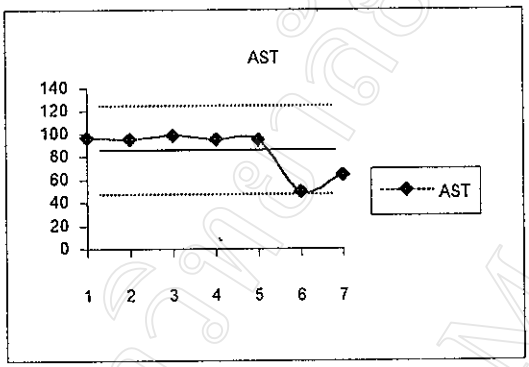
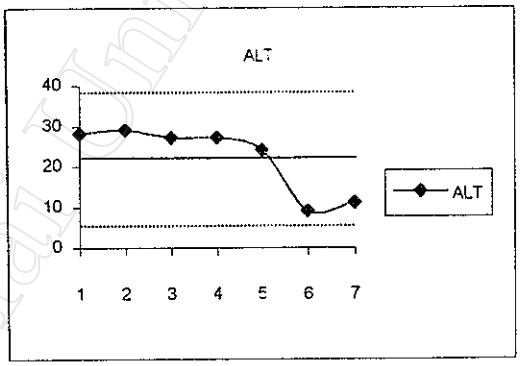
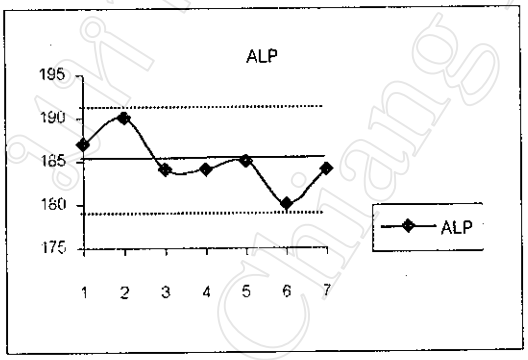
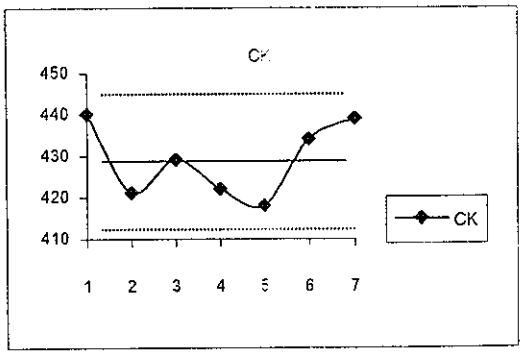


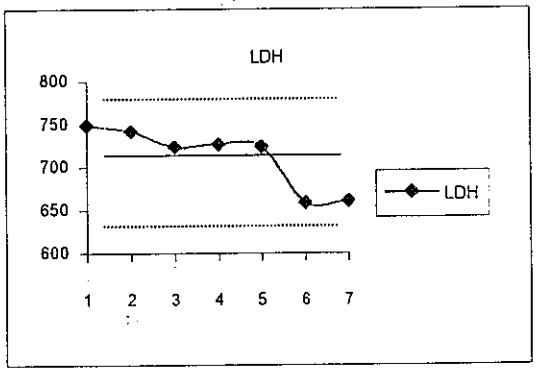


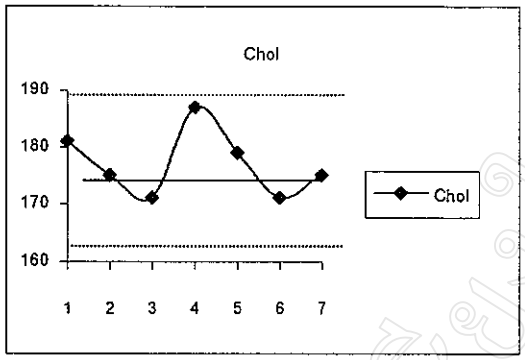


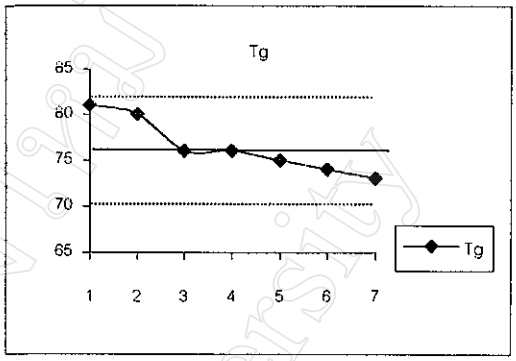


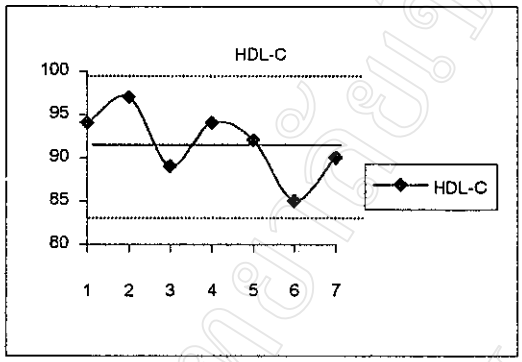


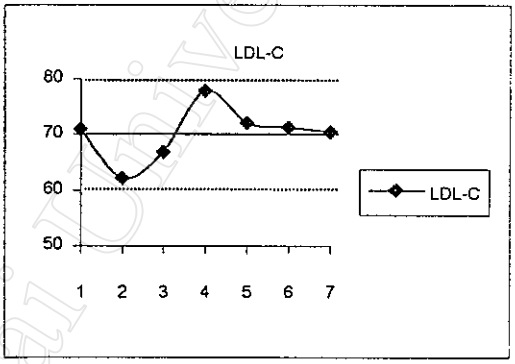




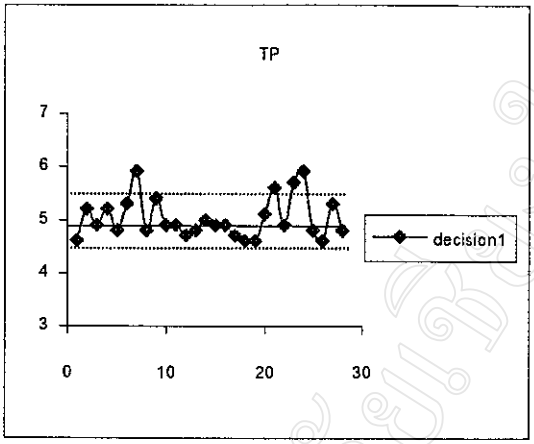


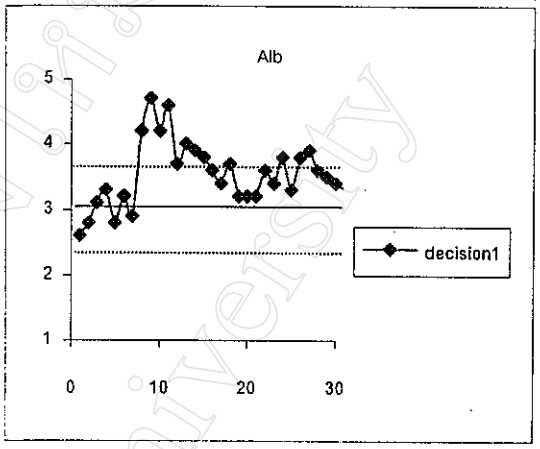


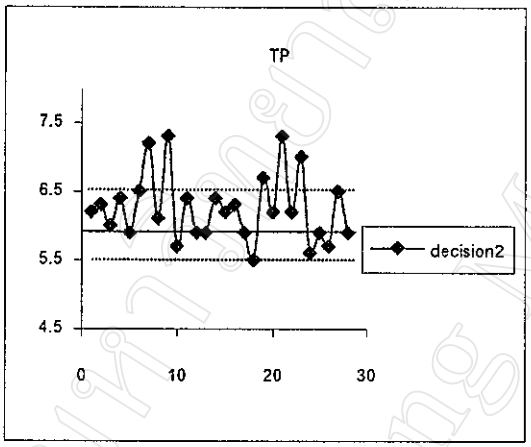


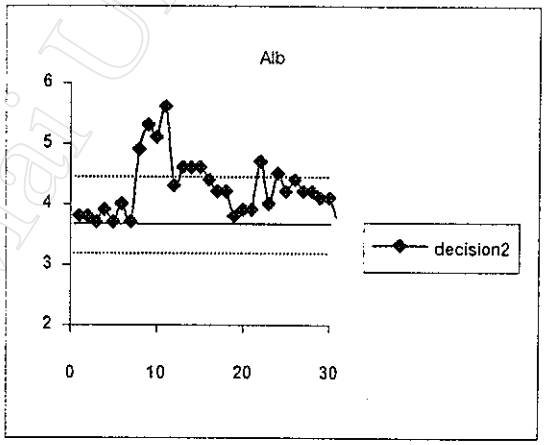


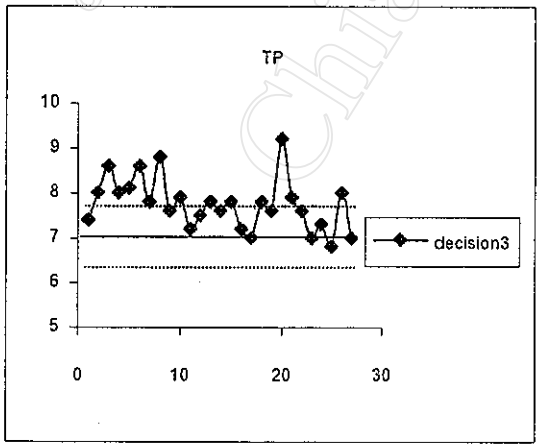
IV. Precision of Beckman CX-5 Autoanalyzer in analyzing protein, enzyme and lipid component concentrations (or activities) (analytical RCV-K).

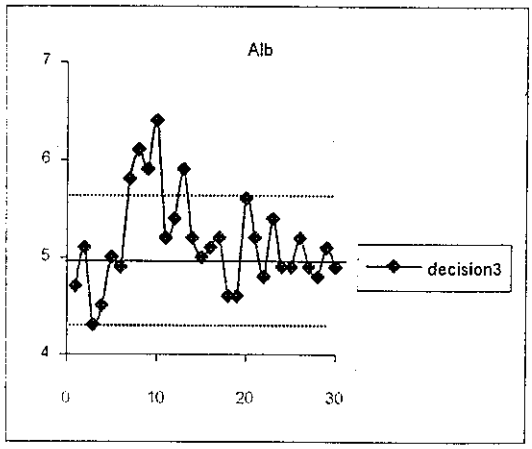


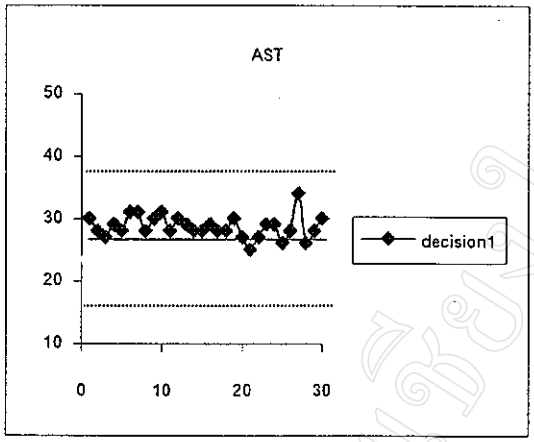


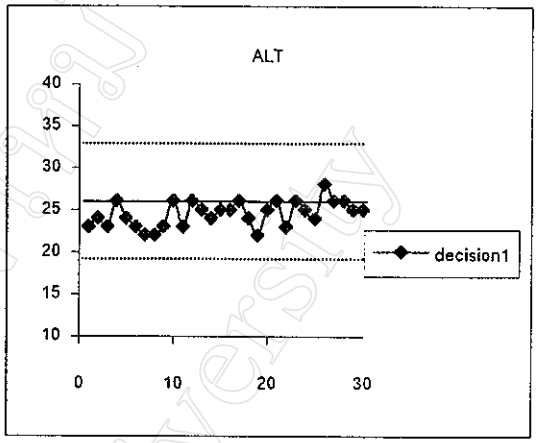









AST
50
40
30
20
10
0
10
20
30
decision1


ALT
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35
30
25
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15
10
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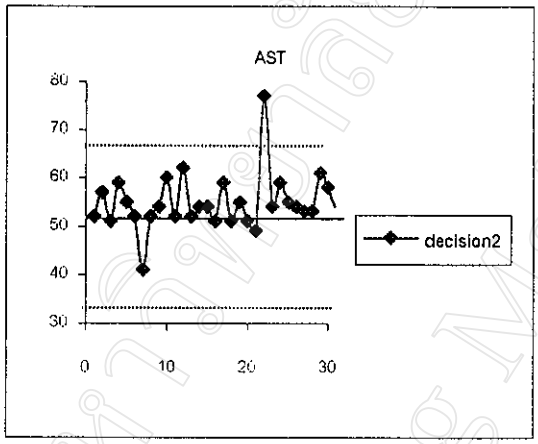
10
20
30
decision1


AST
80
70
60
50
40
30
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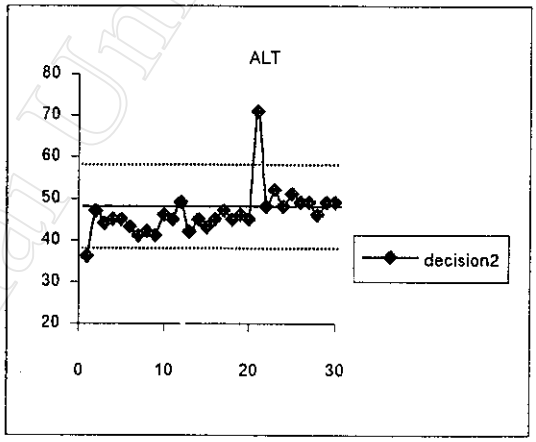
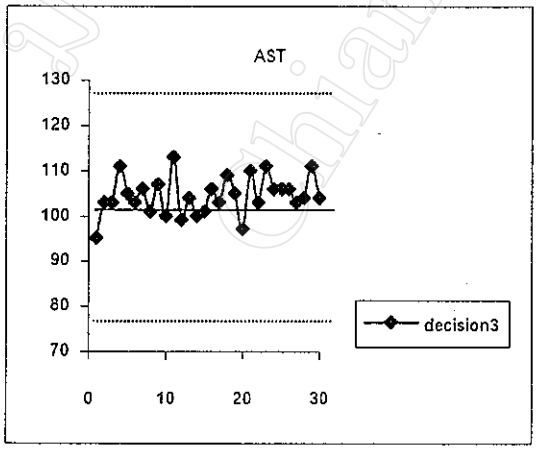
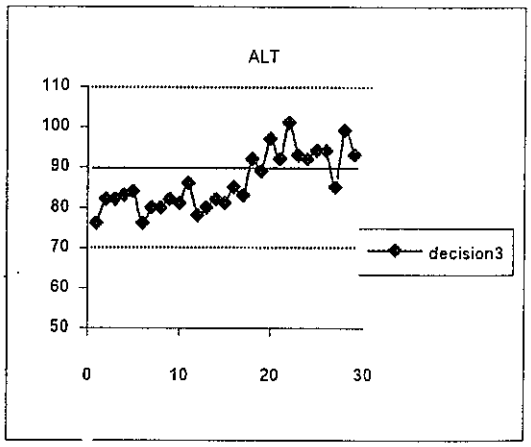
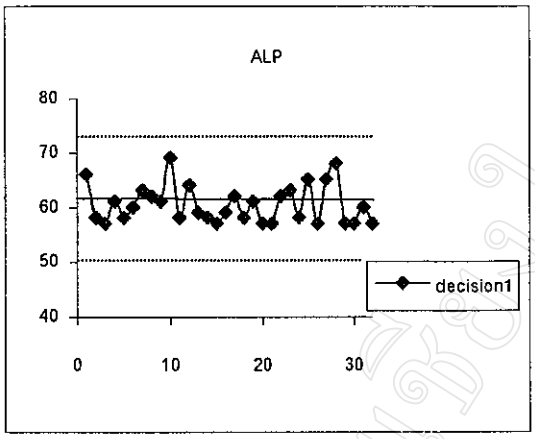
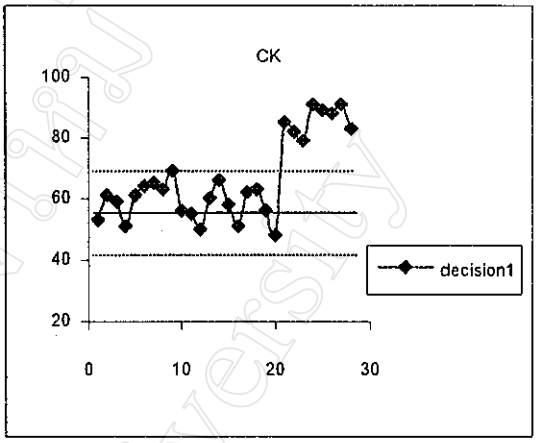
10
20
30
decision2


ALT
80
70
60
50
40
30
20
0
10
20
30
decision2


AST
130
120
110
100
90
80
70
0
10
20
30
decision3


ALT
110
100
90
80
70
60
50
0
10
20
30
decision3


ALP
80
70
60
50
40
0
10
20
30
decision1


CK
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60
40
20
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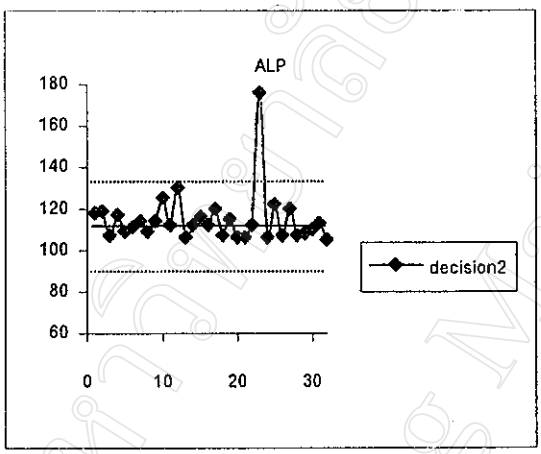
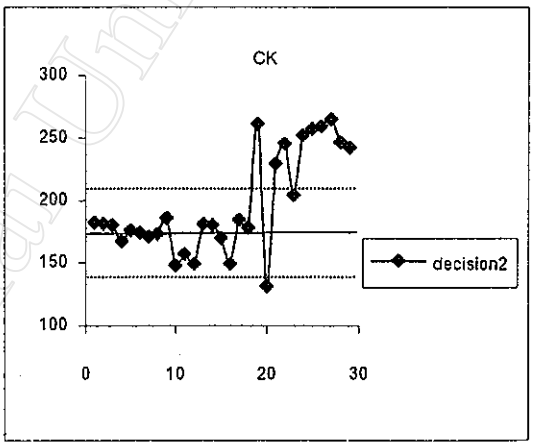
10
20
30
decision1


ALP
180
160
140
120
100
80
60
0
10
20
30
decision2


CK
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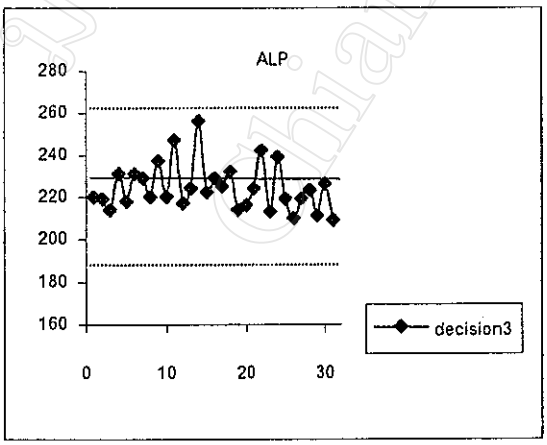
250
200
150
100
0
10
20
30
decision2


ALP
280
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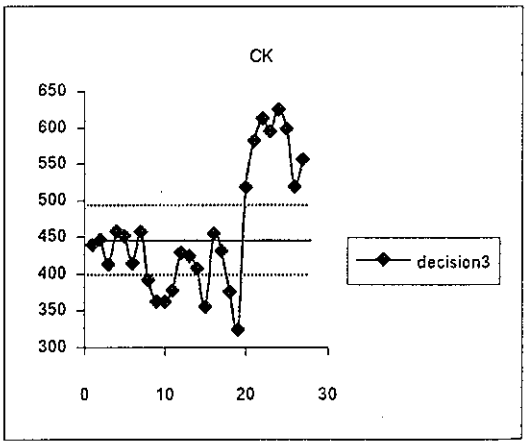
220
200
180
160
0
10
20
30
decision3


CK
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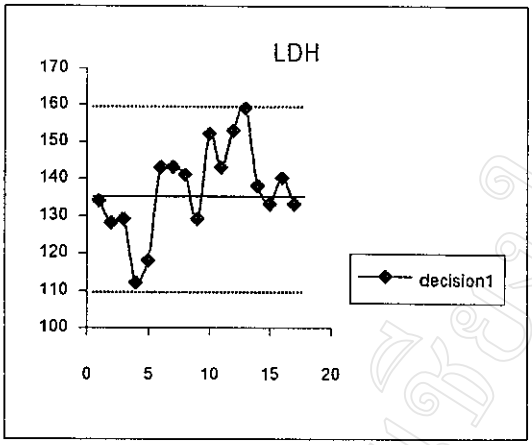
600
550
500
450
400
350
300
0
10
20
30
decision3


LDH
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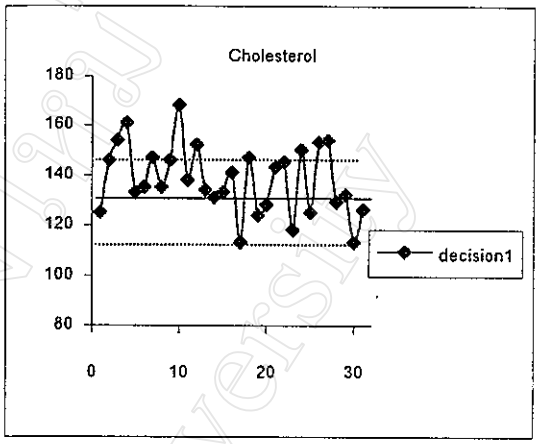
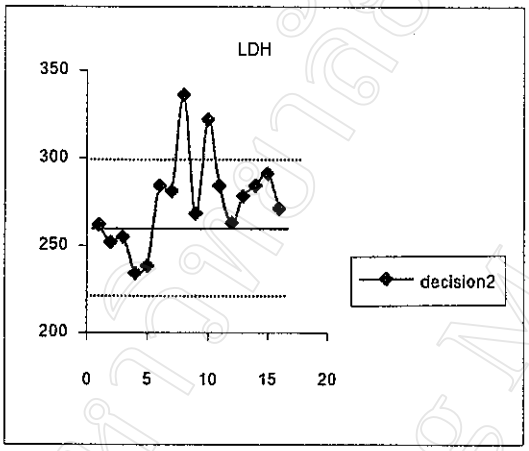
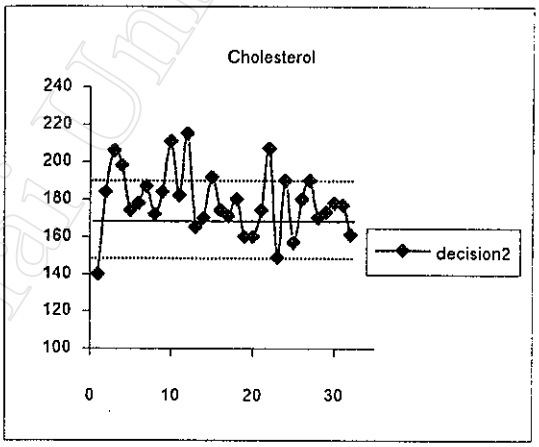
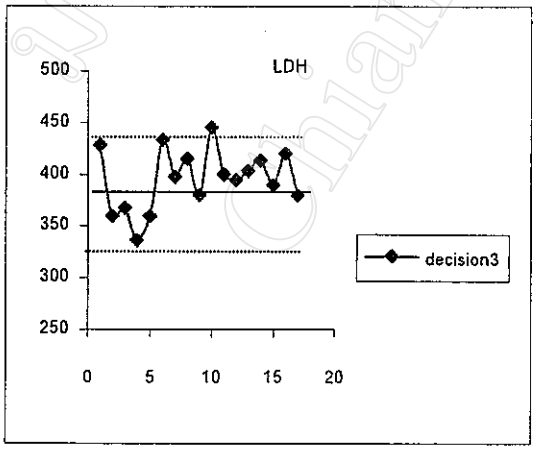
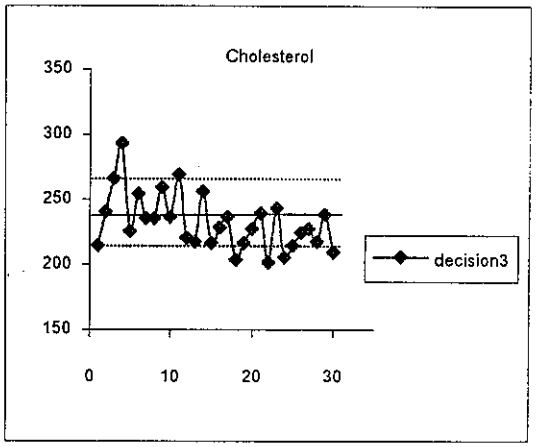
160
150
140
130
120
110
100
0
5
10
15
20
decision1

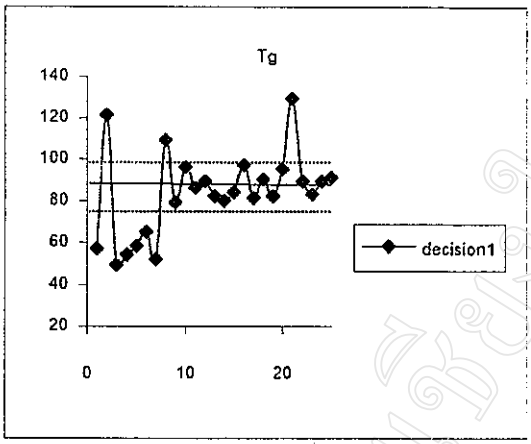

Cholesterol
180
160
140
120
100
80
0
10
20
30
decision1

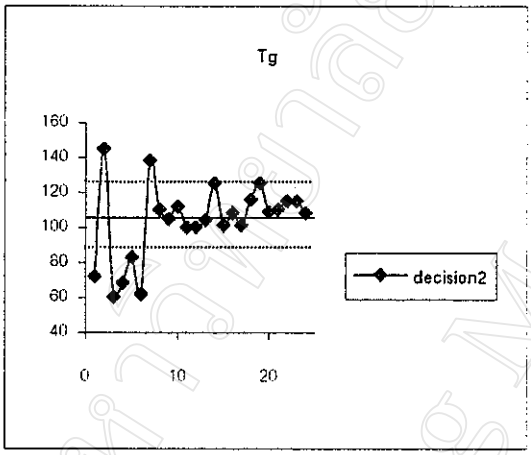

LDH
350
300
250
200
0
5
10
15
20
decision2

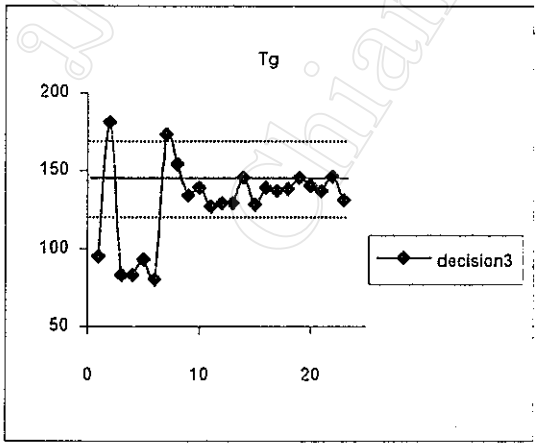

Cholesterol
240
220
200
180
160
140
120
100
0
10
20
30
decision2


LDH
500
450
400
350
300
250
0
5
10
15
20
decision3


Cholesterol
350
300
250
200
150
0
10
20
30
decision3







V. The percentage of moisture content of lyophilized sera.

Vial	Initial weight (g)	remain weight (g)	loss in weight (g)	%moisture content
Level I bovine control serum	0.827	0.820	0.007	0.850
Level II bovine control serum	0.829	0.812	0.017	2.050
Level I human control serum	0.822	0.815	0.007	0.850
Level II human control serum	0.842	0.828	0.014	1.660

VI. Stability of two level lyophilized bovine control sera.

Details of accelerated temperature testing of two level lyophilized bovine control sera.

(1.) Stability of level I bovine control serum containing 8.5% saccharose kept at $4^{O}C$.*

TEST	Cont r ol	1 D **	%R	7 D	%R	15 D	%R	30 D	%R	45 D	%R	60 D	%R
TP	5.6 ± 0.53	5.3 ± 0.38	100	5.3 ± 0.57	99.6	5.9 ± 0.07	110	5.4 ± 0.07	101	5.4 ± 0.0	102	6 ± 0.0	108.1
Alb	2.9 ± 0.62	2.7 ± 0.60	100	3.0 ± 0.07	108	3.0 ± 0.14	110	2.8 ± 0.14	103	3.0 ± 0.2	110	2.8 ± 0.1	94.02
AST	75 ± 3.42	108 ± 10.7	100	77 ± 21.2	71.3	83 ± 4.2	76.9	89 ± 1.41	83	73 ± 1.4	67.6	82 ± 2.8	109.3
ALT	47 ± 7.07	65 ± 7.2	100	50 ± 2.8	76.9	34 ± 13.4	52.3	38 ± 0.71	59	22 ± 0.7	33.8	20 ± 0.0	42.55
ALP	82 ± 4.1	49 ± 12.2	100	55 ± 6.4	111	59 ± 0.0	121	58 ± 2.12	118	57 ± 0.7	116	59 ± 1.4	71.62
CK	243 ± 13.1	331 ± 21.9	100	373 ± 93.3	113	367 ± 171	111	474 ± 5.66	143	469 ± 0.0	142	478 ± 35.4	196.7
LDH	187 ± 16.3	143 ± 8.1	100	142 ± 0.0	99.3	151 ± 1.4	106	144 ± 2.83	101	137 ± 0.0	95.8	152 ± 3.5	81.02
TC	131 ± 8.54	105 ± 4.5	100	113 ± 9.9	108	97 ± 7.1	92.6	89 ± 2.12	85	104 ± 3.5	99.2	90 ± 18.4	68.97
Tg	132 ± 14.4	85 ± 14.7	100	79 ± 2.1	92.9	78 ± 4.2	92.3	81 ± 1.41	96	77 ± 1.4	91.1	92 ± 2.8	69.76
HDL-C	18 ± 1.07	23 ± 4.4	100	25 ± 2.1	106	27 ± 19.8	116	29 ± 0.71	123	28 ± 0.0	121	38.5 ± 2.1	220
LDL-C	87 ± 7.59	65 ± 4.7	100	73 ± 11.6	113	95 ± 46.1	147	44 ± 1.13	68	62 ± 0.0	95.8	33.1 ± 16.8	38.21

Control = reconstituted control serum without saccharose.

* Data expressed as $\overline{X}$ + SD of the triplicate analyses.

** concentration at first day of starting experiment, D= storage duration(days).

%R = % remaining concentration or activity in the shade was not accepted for stability.

(2.) Stability of level I bovine control serum containing 8.5% saccharose kept at 20°C.*

TEST	Cont r ol	1 D**	%R	7 D	%R	15 D	%R	30 D	%R	45 D	%R	60 D	%R
TP	5.6 ± 0.53	5.3 ± 0.38	100	5.3 ± 0.49	99.1	5.9 ± 0.07	110	5.4 ± 0.0	102	5.3 ± 0.0	100	5.95 ± 0.2	112.3
Alb	2.9 ± 0.62	2.7 ± 0.60	100	3.0 ± 0.14	111	2.9 ± 0.14	107	2.7 ± 0.1	100	3.0 ± 0.0	111	2.8 ± 0.1	101.9
AST	75 ± 3.42	108 ± 10.7	100	83 ± 2.83	76.9	71 ± 4.24	65.7	62 ± 8.4	57.4	53 ± 7.1	49.1	69 ± 1.4	63.89
ALT	47 ± 7.07	65 ± 7.2	100	40 ± 5.66	61.5	18 ± 10.6	27.7	24 ± 0.7	36.9	22 ± 1.4	33.8	14 ± 0.0	21.54
ALP	82 ± 4.1	49 ± 12.2	100	56 ± 6.36	113	59 ± 2.83	120	59 ± 0.7	119	60 ± 0.7	122	62 ± 2.8	126.5
CK	243 ± 13.1	331 ± 21.9	100	354 ± 98.9	107	329 ± 145	99.2	428 ± 1.4	129	439 ± 0.0	133	408.5 ± 9.2	123.4
LDH	187 ± 16.3	143 ± 8.1	100	140 ± 5.66	97.9	151 ± 2.83	106	148 ± 3.5	103	137 ± 0.0	95.8	149.5 ± 3.5	104.5
TC	131 ± 8.54	105 ± 4.5	100	106 ± 0.71	100	98 ± 2.12	92.9	91 ± 0.7	86.2	99 ± 0.7	94.3	107 ± 8.5	101.9
Tg	132 ± 14.4	85 ± 14.7	100	80 ± 0.00	94.1	82 ± 0.71	95.9	79 ± 2.1	92.4	81 ± 2.1	95.3	86.5 ± 0.7	101.8
HDL-C	18 ± 1.07	23 ± 4.4	100	24 ± 0.00	104	29 ± 12.0	124	18 ± 0.7	76.1	28 ± 0.0	122	53 ± 8.5	230.4
LDL-C	87 ± 7.59	65 ± 4.7	100	66 ± 0.71	101	90 ± 42.2	138	57 ± 0.9	88.2	55 ± 0.0	84.6	36.7 ± 0.1	56.46

Control = reconstituted control serum without saccharose.

* Data expressed as $\bar{X}$ + SD of the triplicate analyses.

** concentration at first day of starting experiment, D= storage duration(days).

%R = % remaining concentration or activity in the shade was not accepted for stability.

(3.) Stability of level I bovine control serum containing 8.5% saccharose kept at 45°C.*

TEST	Cont r ol	1 D**	%R	7 D	%R	15 D	%R	30D	%R	60 D	%R
TP	5.6 ± 0.53	5.3 ± 0.38	100	5.3 ± 0.57	100	5.8 ± 0.14	109.4	5.4 ± 0.07	101	6.0 ± 0.0	113
Alb	2.9 ± 0.62	2.7 ± 0.60	100	3.0 ± 0.07	109	2.9 ± 0.14	107.4	2.7 ± 0.07	98.1	2.7 ± 0.2	98.1
AST	75 ± 3.42	108 ± 10.7	100	79 ± 7.1	73.1	63 ± 7.1	58.3	55 ± 0.71	50.9	33 ± 0.7	30.6
ALT	47 ± 7.07	65 ± 7.2	100	34 ± 0.0	52.3	22 ± 14.1	33.8	22 ± 0.71	33.8	13 ± 0.7	20
ALP	82 ± 4.1	49 ± 12.2	100	57 ± 6.4	115.3	59 ± 2.8	120.4	56 ± 1.41	114	61.5 ± 0.7	126
CK	243 ± 13.1	331 ± 21.9	100	390 ± 93.3	117.8	333 ± 140	100.6	448 ± 2.83	135	324.5 ± 14.8	98
LDH	187 ± 16.3	143 ± 8.1	100	143 ± 2.1	99.7	155 ± 5.7	108.4	148 ± 0.71	103	157 ± 2.8	110
TC	131 ± 8.54	105 ± 4.5	100	101 ± 0.7	95.7	101 ± 9.2	95.7	96 ± 4.95	91	120.5 ± 16.3	115
Tg	132 ± 14.4	85 ± 14.7	100	79 ± 2.1	92.4	81 ± 0.7	94.7	78 ± 0.00	91.8	86 ± 1.4	101
HDL-C	18 ± 1.07	23 ± 4.4	100	26 ± 8.5	113.0	30 ± 22.6	130.4	20 ± 0.71	84.8	70 ± 18.4	304
LDL-C	87 ± 7.59	65 ± 4.7	100	59 ± 7.4	90.5	100 ± 51.5	154.5	60 ± 4.24	92.9	33.3 ± 2.4	51.2

Control = reconstituted control serum without saccharose.
* Data expressed as $\bar{X}$ + SD of the triplicate analyses.
** concentration at first day of starting experiment, D= storage duration(days).
%R = % remaining concentration or activity in the shade was not accepted for stability.

(4.) Stability of level II bovine control serum containing 8.5% saccharose kept at $4^{O}C$.*

TEST	Cont r ol	1 D**	%R	7 D	%R	15D	%R	30D	%R	45 D	%R	60 D	%R
TP	7.1 ± 0.88	6.5 ± 0.67	100	7.0 ± 0.78	106.6	7.2 ± 0.49	109.7	7.1 ± 0.07	108	6.0 ± 0.0	92	7.0 ± 0.1	106.6
Alb	3.0 ± 0.75	2.6 ± 0.74	100	3.1 ± 0.14	118.9	3.0 ± 0.14	115.1	2.8 ± 0.14	107	3.1 ± 0.1	119	3.0 ± 0.1	115.1
AST	115 ± 18	79 ± 13.2	100	120 ± 16.90	151.9	127 ± 9.9	160.8	131 ± 9.90	166	122 ± 5.7	154	150 ± 8.5	189.9
ALT	70 ± 5.7	54 ± 4.3	100	52 ± 5.66	96.3	48 ± 8.49	88.89	48 ± 2.12	88.9	58 ± 3.5	107	52 ± 2.8	96.3
ALP	192 ± 11.1	203 ± 5.0	100	200 ± 12.73	98.36	192 ± 4.95	94.18	184 ± 0.71	90.2	187 ± 2.1	92	198 ± 6.4	97.13
CK	422 ± 53.7	218 ± 128.6	100	233 ± 94.05	106.6	311 ± 68.6	142.4	513 ± 2.12	235	482 ± 0.0	221	383 ± 50.9	175.6
LDH	578 ± 64.2	462 ± 161.1	100	522 ± 57.98	113	588 ± 3.54	127.1	586 ± 2.12	127	564 ± 0.0	122	614 ± 4.2	132.9
TC	218 ± 3.96	213 ± 5.8	100	218 ± 1.41	102.2	211 ± 25.5	98.94	201 ± 2.12	94	211 ± 2.8	98.9	229 ± 5.7	107.4
Tg	141 ± 4.84	161 ± 4.4	100	164 ± 9.19	101.3	155 ± 7.78	95.69	148 ± 1.41	91.7	151 ± 2.1	93.5	160 ± 4.2	99.09
HDL-C	66 ± 7.99	66 ± 8.5	100	79 ± 12.02	119.8	74 ± 20.5	112.2	59.5 ± 0.71	90.8	73 ± 0.0	111	93 ± 4.2	141.9
LDL-C	124 ± 8.16	112 ± 15.5	100	107 ± 15.27	95.28	195 ± 128	173.6	111 ± 2.55	99.4	110 ± 0.0	98.1	104 ± 9.1	92.78

Control = reconstituted control serum without saccharose.

* Data expressed as $\bar{X}$ + SD of the triplicate analyses.

** concentration at first day of starting experiment, D= storage duration(days).

%R = % remaining concentration or activity.

(5.) Stability of level II bovine control serum containing 8.5% saccharose kept at $20^{O}C$.*

TEST	Cont r ol	1 D**	%R	7 D	%R	15D	%R	30D	%R	45 D	%R	60 D	%R
TP	7.1 ± 0.88	6.5 ± 0.67	100	7.0 ± 0.58	106.9	7.4 ± 0.40	113.1	7.25 ± 0.2	112	6.1 ± 0.0	93.8	6.85 ± 0.1	105.4
Alb	3.0 ± 0.75	2.6 ± 0.74	100	3.1 ± 0.06	117.3	3.1 ± 0.08	119.2	2.95 ± 0.1	113	3.1 ± 0.1	119	2.95 ± 0.1	113.5
AST	115 ± 18	79 ± 13.2	100	111 ± 6.20	140.5	124 ± 8.6	157	117 ± 8.7	148	107 ± 11.5	135	126 ± 14.1	159.5
ALT	70 ± 5.7	54 ± 4.3	100	57 ± 1.41	105.6	49 ± 4.2	90.74	47 ± 1.4	87	47 ± 4.2	87	53 ± 7.1	98.15
ALP	192 ± 11.1	203 ± 5.0	100	186 ± 7.72	91.24	185 ± 5.5	91.12	179 ± 3.5	87.8	182 ± 0.7	89.5	189 ± 9.2	92.72
CK	422 ± 53.7	118 ± 128.6	100	260 ± 10.66	219.7	306 ± 68.1	259.1	494 ± 16.3	418	453 ± 0.0	384	403 ± 26.9	341.2
LDH	578 ± 64.2	462 ± 161.1	100	562 ± 14.63	121.6	614 ± 7.7	132.8	596 ± 19.8	129	575 ± 0.0	124	603 ± 11.3	130.5
TC	218 ± 3.96	213 ± 5.8	100	216 ± 5.38	101.4	219 ± 11.3	102.6	206 ± 6.4	96.3	208 ± 3.5	97.5	219 ± 9.2	102.4
Tg	141 ± 4.84	162 ± 4.4	100	162 ± 6.24	100	160 ± 5.5	98.92	154 ± 2.1	95	154 ± 1.4	95.4	160 ± 5.7	99.07
HDL-C	66 ± 7.99	66 ± 8.5	100	76 ± 8.96	115.6	78 ± 16.0	118.3	63 ± 1.4	96.2	69 ± 0.0	105	82.5 ± 9.2	126
LDL-C	124 ± 8.16	112 ± 15.5	100	108 ± 11.02	96.52	200 ± 99.7	178.7	112 ± 4.5	99.7	107 ± 0.0	95.5	104 ± 1.1	92.77

Control = reconstituted control serum without saccharose.

* Data expressed as $\bar{X}$ + SD of the triplicate analyses.

** concentration at first day of starting experiment, D= storage duration(days).

%R = % remaining concentration or activity.

(6.) Stability of level II bovine control serum containing 8.5% saccharose kept at 45°C.*

TEST	Cont r ol	1D**	%R	7D	%R	15D	%R	30 D	%R	45 D	%R	60 D	%R
TP	7.1 ± 0.88	6.5 ± 0.67	100	7.0 ± 0.64	106.9	6.8 ± 0.41	104.6	7.4 ± 0.1	113	6.3 ± 0.0	96.9	6.8 ± 0.0	104.6
Alb	3.0 ± 0.75	2.6 ± 0.74	100	3.1 ± 0.06	117.3	2.8 ± 0.10	108.7	3.0 ± 0.1	113	3 ± 0.1	115	2.85 ± 0.1	109.6
AST	115 ± 18	79 ± 13.2	100	104 ± 10.3	131.6	107 ± 8.1	135.4	99 ± 8.7	125	103 ± 4.8	130	108 ± 9.2	136.7
ALT	70 ± 5.7	54 ± 4.3	100	58 ± 2.8	107.4	49 ± 1.4	90.7	44 ± 2.8	81.5	40 ± 5.7	74.1	37 ± 0.7	68.52
ALP	192 ± 11.1	203 ± 5.0	100	187 ± 8.8	92.1	169 ± 10.1	82.9	175 ± 0.7	85.8	178 ± 1.1	87.6	183 ± 3.5	89.77
CK	422 ± 53.7	118 ± 128.6	100	265 ± 13.2	224.4	281 ± 67.0	237.5	396 ± 17.0	335	402 ± 0.0	340	362 ± 9.9	306.5
LDH	578 ± 64.2	462 ± 161.1	100	569 ± 8.3	123	565 ± 41.2	122.2	605 ± 4.2	131	568 ± 0.0	123	605 ± 0.7	130.8
TC	218 ± 3.96	213 ± 5.8	100	215 ± 4.4	100.6	201 ± 6.8	94	193 ± 7.1	90.5	200 ± 2.5	93.8	223 ± 21.2	104.5
Tg	141 ± 4.84	162 ± 4.4	100	161 ± 4.4	99.7	142 ± 8.2	87.6	148 ± 8.5	91.6	145 ± 0.7	89.8	153 ± 2.1	94.43
HDL-C	66 ± 7.99	66 ± 8.5	100	71 ± 7.4	108.0	62 ± 6.5	94.7	59 ± 2.1	89.3	68 ± 0.0	104	84.5 ± 14.8	129
LDL-C	124 ± 8.16	112 ± 15.5	100	112 ± 6.5	99.5	175 ± 77.5	155.8	105 ± 10.9	93.6	106 ± 0.0	94.6	108 ± 5.9	96.34

Control = reconstituted control serum without saccharose.

* Data expressed as $\bar{X}$ + SD of the triplicate analyses.

** concentration at first day of starting experiment, D= storage duration(days).

%R = % remaining concentration or activity in the shade was not accepted for stability.

VII. Cost of preparation.

Method for calculation of standard added in the control serum. Demonstration of type and costs of chemical which used to add in control serum.

[1.] Demonstration of type and costs of chemical which used to add in control serum.

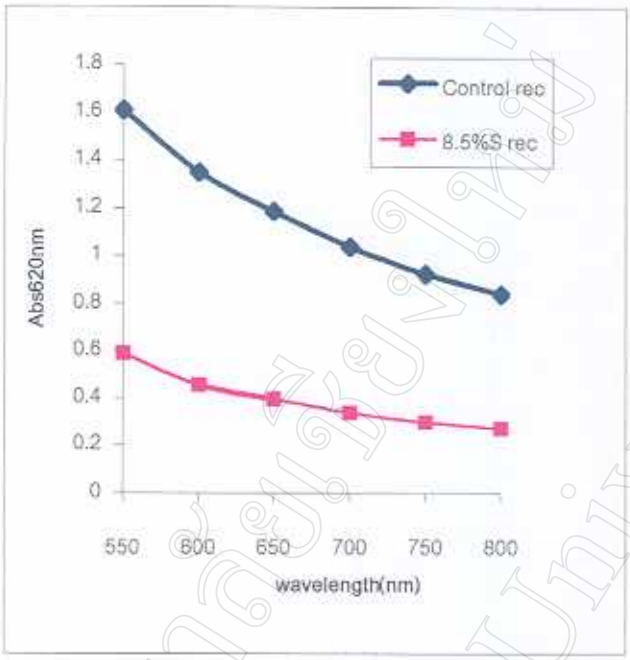
Analyte	Chemical used	Cat No. (Sigma, 1998)	Size	Cost, $ / pack	Baht /pack	Cost, Baht per unit*
TC	Self prep.	-	approx./ 500 mg.	-	3,457.50	6,915.-
Albumin	Bovine	A-3425	100 g	124.10	7,446.-	74.46
ALP	Self prep.	-	97 U	-	75.-	0.77
AST	Self prep.	-	120 U	-	75.-	0.625
ALT	Porcine heart	G-8255	2,000 U	185.30	11,118.	5.56
CK	Bovine heart	C-7886	1000	68.80	3,100.-	3.10
LDH	Porcine heart	ICN-151532	1000	54.40	2,450.-	2.45

* Cost/Unit; g or unit of enzyme

[2.] Cost of chemical added per batch (4 liters).

Analyte	Level I		Level II	
	Quantity added/4 L	Cost (baht)	Quantity added/4 L	Cost (baht)
Cholesterol	20 mg	138.40	0.432 g	2,987.28
Albumin	44.08 g	3,300.-	32 g	2,382.72
ALP	106.4 U	204.82	221.9 U	170.87
AST	NA	-	46.08 U	28.80
ALT	16 U	88.96	114 U	800.64
CK	0.186 U	570.40	176 U	545.60
LDH	128 U	313.60	976 U	2,450.-
SUM	-	5,230.-	-	9,365.91

VIII. Determination of clarity of control serum.



(1.) The clarity of reconstituted lyophilized control sera with and without 8.5% saccharose measured by scanning the absorbance from 800 to 550 nm in a Shimadzu UV-160A Spectrophotometer.

IX. Demonstration of 23 chemical compositions in bovine control serum containing excipient.

Test	pool	T5%	T10%	S5%	S10%	M5%	M10%	D1%	D5%
Glucose	68	70	70	65	63	68	72	66	61
BUN	24	24	22	23	22	23	22	23	23
Creatinine	3.9	3.7	3.5	3.6	3.5	3.6	3.5	3.7	3.7
Uric acid	7.7	7.5	7.3	7.4	7.2	7.3	7.3	7.6	7.4
TP	9.8	10.4	9.5	9.8	9.6	10.2	9.9	8.4	10.2
Albumin	5.2	4.8	4.8	4.8	4.8	4.9	4.9	5	4.9
AST	198	148	137	149	141	140	126	151	161
ALT	36	51	45	51	42	42	39	45	51
ALP	121	114	110	113	108	112	110	112	112
CK	244	243	232	244	227	218	237	233	232
LDH	710	674	648	701	649	665	655	699	692
TC	241	234	223	230	222	225	219	235	229
Tg	ND	ND	ND	ND	ND	ND	ND	ND	ND
HDL-C	53.9	50.6	60.5	50.6	78.1	49.5	64.9	50.6	44
LDL-C	ND	ND	ND	ND	ND	ND	ND	ND	ND
Na	184	185	186	185	187	181	185	181	182
K	6.54	6.52	6.5	6.57	6.58	6.44	6.56	6.46	6.3
Cl	122	121	120	121	120	120	119	122	121
TCO2	18	18	18	18	17	19	18	18	16
Ca	10.2	10.1	10	10.2	10.1	10.2	10.3	10.1	10.2
Mg	3.1	5.3	2.9	2.9	2.8	2.9	2.8	2.9	2.9
PO4	7.8	7.9	7.3	7.6	7.4	7.9	7.8	7.7	7.7
Fe	194	194	195	196	194	196	197	197	196

ND=Not determined, T=Trehalose, S=Saccharose, M=Mannitol, D=Dextran, %=W/V

X. Definition and explaination of glass transition temperature *(Tg)*.

When a polymer is cooled below its glass transition temperature it becomes hard and brittle-like glass.

The relation of *Tg* and freezing-drying process (lyophilization).

Freezing is the first important process that proteins undergo during formulation. Protein solutions become thermodynamically unstable when supercooled below 0°C. Ice nucleation begins at around –15 to -20°C, and growth of ice crystals proceeds according to cooling conditions. Amorphous phases consisting of protein molecules and other solutes as well as unfrozen water are formed as growth of ice crystals proceeds in the freezing solutions.

Growth of ice crystals increases the concentrations of solutes in the amorphous phase. The final concentrations of solutes in the phase depend on the cooling rate and the final temperature rather than on the initial concentration when rapid freezing is used, as is the case with immersion in liquid nitrogen. This occurs only with very small volumes of bulk liquids, and may not hold for real-time cooling. The final concentrations usually increase when larger volumes of solutions are slowly frozen on the shelves of freeze-dryers. At sufficiently low temperatures, the viscosity of the amorphous phase increases markedly in response to small decreases in temperature, and the concentrated viscous phase changes to a solid-like glass. This change, known as "glass transition", plays an important role in the stabilization of proteins during the freeze-drying process. Glass transition affects protein stability not only during freezing but also during subsequent freeze-drying and storage processes. At temperatures below the glass transition temperature (*Tg*), no further crystallization of ice occurs and the frozen solutions become "partially crystallized glass", consisting of both the solid-like glass phase and the ice crystal phase (Izutsu and Yoshioka, 1995).

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