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TWIN TEST APPLICATION OF TOTAL PROTEİN AND ALBUMIN TO HITACHI 705 AUTOANALYZER

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SUMMARY :

We applied a new twin test for serum albümin and protein assays to the autoanalyzer Hitachi 705. Bromcresol green method was used tor albumin determination an biüret method was used tor total protein determination. First we assayed albumin and after wards biuret reagent sended to albumin-bromcresol green complexes. Later, total protein was determined. Twin test results for the total protein were compaired with single test. The corelation between twin and single test rusults was statistically significant ($y=1.112\ x-0.454$; $r=0.998$).

INTRODUCTION :

It is well known that serum total protein (TP) and albumin (ALB) concentrations can be determined photometrically and that the methods used can be applied to many autoanalyzers (1-7). The principle of Biuret reaction for total protein determination is the measurement of coloured complex of two or more peptide bonds in precense of Cu^{+2} ions in alkaline medium, at 540-560-nm. On the other hand albumin forms a coloured complex with bromcresol green (BCG: 3', 3'', 5', 5'' -tetrabrom-m-cresol sulfonaphtalene). The BCG has an absorption maximum at 423 nm' and its sodium salt gives to absorption peaks at 400 and 612-nm. The colour of BCG solution is yellow at pH 3.8 and blue-green at pH 5.4. The peak at the low wavelength is called acidic peak (8).

The factors influencing the ALB-BCG complex formation include pH, dye content, albumin concentration of the sample, and even total protein content of the sample (8). However, in cilinical chemistry laboratories the optimal conditions for ALB determination by BCG method can be achieved (5-8).

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In random access analyzers, any two analytes having no interference with each other with many respects can be analyzed in one reaction cuvette by using the same sample but corresponding, differeent reagnets. Such tests are called twin tests are in the case of BUN/GLU, TG/CHOL, and GOT/GPT in Hitachi 705 705 applications. Twin test provides several advantages, including higher test capacity, lower sample and reagent volume, and higher test output. For this purpose, we have tried to apply the twin measurement of ALB and TP to Hitachi 705 Autoanalyzer, since BCG and biuret reagents form different complexes at different pHs and give absorbtion peaks at different wavelengths.

MATERIALS AND METHODS

Total protein and albumin determinations were made in control sera (Precinorm U, Lot No 154290; Precinorm U, Lot No 171735; precipath U, Lot No 153 225 from Boehringer, Mannheim) and in serum pools with high and low protein contens. The serum pool of low levels of protein and albumin was obtained from hypoproteinemic patients, and the high-protein serum pool was made by adding human albumin to the normal pooled serum.

Reagents: BCG: Succinate buffer, 75 nmol /L pH 4.2;
bromcresol green, 0.15 mmol/L; and Brij 35.

BIURET: NaOH, 0.20 mol/L; K-Na tartarate 52 mmol/L; potassium iodide, 30 mmol/L; and copper sulfate, 12 mmol/L.

The calibration of the autoanalyzer (Hitachi 705, Hitachi/BM) was made with calibration serum (Lot No 156572 from Boehringer, Mannheim). Then the following chemistry parmeteres for twin ALB/TP test were entered:

Test Code	2 (ALB)	32(TP)
Assay code	1 (EP)	1(EP)
Sample volume	3	3
R, volume (BCG)	350-No	350-No
R2 volume (BIURET)	1-1-No	350-No
Wavelength 1	700 nm	700 nm
Wavelength 2	600 nm	546 nm
Std. Abs. Allowance	10 %	10 %
Test code in channel setting	2-32	

Chemicals were obtained from merck Co. Darmstat.

Wavelegth scanning was carried out with spectrophotometer (LKB-4053 Ultrospec K). For the following mixtures the scanning was made from 360 to 660 nm:

1— BCG Reagent. (Blank)

3— BCG reag + sample (single ALB test)

3— BCG reag + biuret reag. (Blank, twin ALB/TP)

4— BCG reag + biuret reag + sample (Twin ALP/TM test)

5— Biuret reag + sample (Single TP test)

RESULT AND DISCUSSION:

The results of wavelength scanning of biuret reagent, BCG reagent, BCG plus biuret, biuret plus serum, BCG plus serum, and BCG plus serum plus biuret are shown in Table 1. and Fig 1. As seen, in reaction medium ALB-BCG complex has two peaks: (514 nm (1.808 A) and 623 nm (1.345A), whereas the peak of Biuret plus serum is at 562 nm as 0.349 A. On the other hand, BCG plus serum pulus biuret mixture has two different peaks at 417 nm (1.525)A) and 626 nm (1.023 A (Fig.1.). In addition, in BCG -Biuret mixture, the acidic peak of BCG has shifted to 397 nm and there is an increase in other peaks, amplitudes.

Table I. Wavelength scanning between 350-650 nm of different mixtures.

Mixture	Wavelength of max. abs (ABS) (nm)	ABS at 546 nm
Biuret	—	0.100
BCG	423 and 620 (1.675 and 0.262)	0.150
Biuret + BCG	397 and 619 (0.743 and 1.887)	0.690
Biuret + serum	562 (0.349)	0.539
BCG + serum	414 and 623 (1.808 and 1.334)	0.600
BCG + şerum+Biuret	417 and 626 (1.252 and 1.023)	0.470

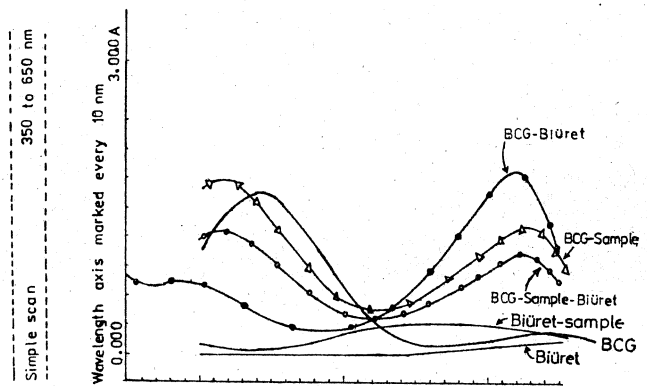

Fig : 1

After one channel for ALB, on channel tor TP, and one channel for twin ALB/TP were set, 5 different kinds of control sera and serum pools were-loaded on the Autoanalyzer, 15 test being made for-every kind of sera. The concentraion of the analytes and the measured values are given in Table II.

Table II. Single and Twin Test Results of TP and ALB

Calibration serum, (range)g/dl	n	Single test (x±SE)	Twin test x ± SE	Difference beetwen single and twin test
ALB 3 1 (2 7—3 5)	15	3.09±0.11	3.07±0.12	N.S.x
TP 4 5 (4 2, 4 9)	15	4 97±0 13	5 10±0 11	N.S.
ALB 3.4 (3 1—3 8)	15	3.36±0.13	3.31±0.15	N.S.
TP 5 (4 5—5 4)	15	5 33±0 13	5 40±0 10	N.S.
ALB 3 9 (1 7—3 3)	15	3 87±0 09	3.89±0.13	N.S.
TP 5 9 (4 5—5 3)	15	5 01±0 12	5 15±0 15	N.S.
ALB 7 9 (7 0—8 8)	15	7.87±0 08	7.87±0.09	N.S.
TP 10 5 (9 6—11 7	15	10 36±0 14	10 80±0 24	N.S.
ALB1 92 (1 87,1 99)	15	1 95±0 09	1.87±0 03	N.S.
TP 3 98 (3 84,4 21)	15	4 05±0.09	3 89±0 10	N.S.

(x) N.S. none significant for student test.

The corelation between single and twin test results for TP was statistically significant (y= 1.112x—0.454;-r= 0.998) (Fig. 2). On the other hand, the t-test showed no statistically significant difference between single and twin test results of TP (Table II).

In Hitachi 705 autoanalyzer, BCG reagent was placed in a channel at R_1 position and biuret reagen at R_2 position of the same channel. In this situation, the instrument pipettes the sample, adds R_1 reagent (for measurement of ALB) and adds R_2 reagent (for measurement of TP) to the same reaction cuvvette. It was not possible to determine TP first, since biuret reagent has an alkaline pH, preventing BCG-ALB complex is formed primarily this complex can easily be dissociated in the pH 8.6 of Biuret reagent in order to form Cu-protein complex (8). It is not necassary to discuss the ALB measurement in single and twin tests, since there is no difference between single and twin test with respect to ALB. However, TP must be discussed. At 546 nm and at pH for TP determination, BCG has higher absorbance than Cu-protein complex. However, immediately after R_2 addition, the instrument takes a blank reading for BCG + Biuret + serum. This is a correction. On the other hand, as mentioned earlier. In signifi-

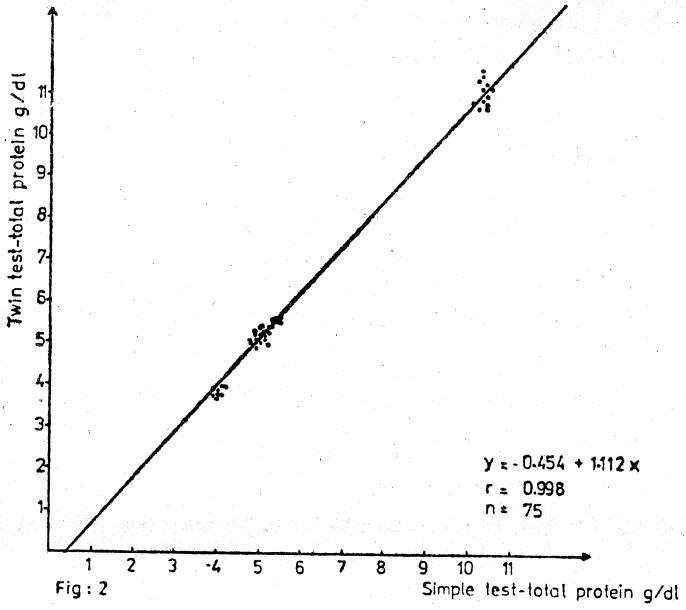

Fig : 2

cant t-test result between single and twin tests and very significand correlation between them show accuracy and presicion of this aplication, suggesitng the twin ALB/TP determination in Hitachi 705 Autoanalyzer.

HİTACHİ 705 OTOANALİZÖRE TOTAL PROTEİN/ALBÜMİN İKİZ TESTİNİN UYGULANMASI

ÖZET

Serum albümin ve total protein tayini için Hitachi 705 otoanalizöre yeni bir ikiz test uyguladık. Albümin tayini, için bromkrezol yeşili, protein tayini için biüret motodları kullanıldı. Önce albümin tayin edildi, sonra albümin-bromkrezol kompleksine biüret reaktifi gönderilerek protein tayin edildi. Bu ikiz test total protein sonuçları, tekli test sonuçları ile karşılaştırıldı. İki test arasındaki doğrusal ilişkinin denklemi $y=1.112x—0.454$ *ve korelasyon katsayısı* $r = 0.998$ *olarak bulundu.*

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