A SENSITIVE AND ECONOMICAL MODIFIED METHOD FOR ESTIMATION OF CEREBROSPINAL FLUID PROTEINS

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ABSTRACT

Cerebrospinal fluid protein estimation is a routine diagnostic tool for various diseases. The proposed modified bromocresol green (BCG) method is applicable to automated as well as manual measurements. Protein concentrations and absorbance at 600 nm are linearly related throughout a wide range of concentrations from 10 mg % to 2000 mg %. Ninety C.S.F. samples were analyzed and results obtained by both the modified BCG method and routine pyrogallol red method were compared. An excellent co-relation was observed ($n = 90$, $r = 0.996$, $P < 0.001$).

KEY WORDS

C.S.F. protein, Modified Bromocresol green (BCG) method, Pyrogallol red method (Kit), SPSS 10.1 Statistical software.

INTRODUCTION

There are a number of methods for measuring proteins in biological fluids. These methods are Colorimetric (1), turbidimetric (2), and electrophoretic (3). Amongst dye binding methods Comassie brilliant blue (4), bromocresol green (B.C.G.) (5) and pyrogallol red [6] are in routine use. However in the case of Comassie brilliant blue the drawback is that after repetitive use over a period of time the dye starts adhering to the side of the cuvettes. This is not the case with BCG and pyrogallol red dyes. In this paper we report a modified method of bromocresol green (manual) and its comparison with pyrogallol red method for C.S.F. protein estimation. The procedure is simple, sensitive and very economical.

MATERIALS AND METHODS

As a standard protein we have used albumin BSA (Loba Chemie India), Succinic acid, Brij 35 (Polyoxyethylene dodecyl ether) bromocresol green, sodium hydroxide were purchased from Himedia India. All reagents were 'Analytical grade'. Pyrogallol red (kit) was purchased from Pointe Scientific Inc. USA.

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The quantities given in the brackets are those used by Spencer and Price (5) in their original BCG method. Dissolve 42 mg (44 mg) of bromocresol green, 600 mg (600 mg) NaOH, 3.57 gm (5.5 gm) succinic acid in 400 ml distilled water. To this add 2.4 gm (490 mg) Brij 35, mix well. The reagent stored in amber colored bottle at 2°C to 8°C was found to be stable for a minimum period of 6 months.

Cerebrospinal fluid samples received in the clinical biochemistry laboratory from Krishna Hospital and Medical Research Center between Aug-2000 to July 2001 period for routine tests were used in the present investigations. Ninety samples chosen randomly were subjected to protein analysis. Protein measurements in these samples were done simultaneously using modified bromocresol green (BCG) method and pyrogallol red method. Bovine serum albumin (50 mg / dl) was used as standard.

PROCEDURES

Modified B.C.G. reagent (manual method) measurement :

50 µl of C.S.F. sample or a standard solution or deionized water as blank were mixed separately with 500 µl of B.C.G. modified reagent and were incubated for 10 mins at 37°C temperature. The absorbance of each was measured at 546 nm against reagent blank using Ebra. Chem. Pro semiautoanalyser.

Pyrogallol red (kit) method

20 µl of deionized water, standard and C.S.F. sample were mixed separately with 1000 µl of pyrogallol red

reagent into labelled tubes for blank, standard and sample respectively. These tubes were incubated for 10 minutes at 37°C. The absorbance of each was measured at 600 nm. The concentrations of protein were calculated by comparing with a standard curve.

Randomly selected 90 samples of C.S.F. were used for protein determination by the modified BCG and pyrogallol red method for comparison. Concentrations of various C.S.F. samples were compared. Co-relation analysis was performed and confirmed by 'paired t test. Data was analyzed statistically by SPSS 10.1 Version software package.

RESULTS AND DISCUSSION

In the present study 90 C.S.F. samples of hospital patients were analyzed by both modified bromocresol green (manual) method and pyrogallol red (Kit) method.

Original BCG method is routinely used for detection of serum albumin concentration. In that method only 10 µl of serum is used and immediately readings are taken. But in modified BCG reagent there is a change in concentration of constituents namely decreased succinic acid and increased Brij 35 concentration and also volume of C.S.F. is increased to 50 µl. After

addition of reagent contents are incubated for 10 min. at 37°C. This appears to help in binding of all proteins present in C.S.F. with dye. Increased concentration of Brij 35 might be stabilizing binding of all proteins with dye.

In our laboratory we were routinely using pyrogallol red (kit) method for determination of C.S.F. proteins, which was quite expensive. The average cost is approximately Rs.20/- per ml whereas the cost of BCG method is around Rs.1.00/- per ml. Therefore we made an attempt to measure the C.S.F. protein by modifying the B.C.G. method for routine use in our laboratory.

The results showed an excellent co-relation between both the methods (Fig. 1) where n = 90, on Y axis mg % of C.S.F. protein by modified B.C.G. method and on X axis mg % of C.S.F. by pyrogallol red (kit) method.

Out of 90 samples analyzed 36 were within normal range by pyrogallol red method. Same samples were simultaneously run for protein measurement with modified B.C.G. method. All 36 samples values obtained by kit method (pyrogallol red) when compared with modified B.C.G. values the mean for pyrogallol red was 28.61 mg % and for B.C.G. it was 28.63 mg % with standard deviation 8.043 and 7.665

Table 1. C.S.F. Protein assay by BCG manual method compared with the pyrogallol red method

Case Summaries

	NORMAL	KIT	MANUAL
normal	N	36	36
	Minimum (mg %)	15.00	17.00
	Maximum (mg %)	45.00	46.00
	Mean	28.6111	28.6389
	Std.Deviation	8.0437	7.6650
	Std. Error of Mean	1.3406	1.2775
non-normal	N	54	54
	Minimum (mg %)	49.00	46.00
	Maximum (mg %)	1510.00	1525.00
	Mean	182.9444	179.4444
	Std.Deviation	229.6570	222.3994
	Std. Error of Mean	31.2524	20.2647
Total	N	90	90
	Minimum	15.00	17.00
	Maximum	1510.00	1525.00
	Mean	121.2111	119.1222
	Std.Deviation	192.9107	187.0754
	Std. Error of Mean	20.3346	19.7195

Table 2. Paired Samples Statistics

	Mean	N	Std.Deviation	Std.Error Mean
Pair KIT	121.2111	90	192.9107	20.3346
1 MANUAL	119.1222	90	187.0754	19.7195

Table 3. Paired Samples Test

	Paired Differences				t	df	Sig (2-tiled)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
				Lower Upper			
Pair 1							
KIT- MANUAL	2.0889	17.4997	1.8446	- 1.5764 5.7541	1.132	89	.261

Fig. 1. CSF protein assay comparison between the BCG manual and the pyrogallol red (kit) method (n=90; r=0.996)

respectively. When these values were compared using 'paired t' test indicate that there was no significant difference, ($P < 0.001$) (Table 1, 2 and 3). It mean the same normal range can be considered even for modified B.C.G. method.

Out of 90 samples of C.S.F. in 54 samples protein concentration were above the normal range (N.R. 15 to 45 mg %). The highest concentration reported with pyrogallol red (Kit) was 1510 mg % where as for the same sample with modified B.C.G. the value was 1525 mg %. Linearity of modified B.C.G. is more than 1000 mg % hence C.S.F. sample can be processed without

dilution, which is required in pyrogallol red method.

Finally we would like to make a few remarks concerning the volume of sample and incubation period as compared to Spencer and Price (5). First, sample volume was increased from 5 μ l to 50 μ l per 500 μ l of reagent, since protein concentration in C.S.F. was very less as compared to serum. Secondly the incubation at 37°C for 10 minutes gives sufficient time for all the proteins present in C.S.F. to bind with B.C.G. instead of taking immediate readings because reaction with albumin is much more rapid than other proteins. Booi (7) and Webster (8) found that globulin fractions reacts significantly with B.C.G. In pathological cases there is an increase in gamma globulin fraction; which appears to binds with BCG at 37°C in 10 minutes and that is why the values obtained by Pyrogallol red excellently correlates with modified BCG method.

Reproducibility studies proved this method to be quite adequate for clinical use. Thus modified B.C.G. method seem to be quite acceptable and economical method for routine use of C.S.F estimation.

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