

SHORT REPORT

Measurement of serum albumin by capillary zone electrophoresis, bromocresol green, bromocresol purple, and immunoassay methods

E B Duly, S Grimason, P Grimason, G Barnes, T R Trinick

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Background/Aims: The introduction of capillary zone electrophoresis (CZE) to this laboratory has highlighted discrepancies in albumin measured on an Abbott Aeroset by bromocresol green (BCG) and that calculated by CZE on the basis of total protein measured by Biuret.

Methods: This study examined differences in albumin estimation by CZE, Abbott Aeroset BCG, and Aeroset bromocresol purple (BCP), and compared these with albumin estimated by Beckman Array immunoassay.

Results: Altman and Bland analysis of results showed a positive bias of BCG with CZE (4.51 g/litre; 95% limits of agreement, 3.77 to 5.26; n = 72) and BCP (3.85 g/litre; 95% limits of agreement, -1.42 to 9.12; n = 72). CZE and BCP agreed closely (0.67 g/litre; 95% limits of agreement, -4.39 to 3.06; n = 72). Analysis of 57 of those samples in which BCG and CZE differed ≥ 5 g/litre showed a positive bias of BCG with immunoassay (8.35 g/litre; 95% limits of agreement, 1.54 to 15.16; n = 57), with good agreement between CZE and immunoassay (-0.44 g/litre; 95% limits of agreement, -2.82 to 1.94; n = 57).

Conclusions: BCP is superior to BCG for the assay of albumin and has replaced BCG as the routine test for albumin in this laboratory.

In Beckman capillary zone electrophoresis (CZE), the separation of serum protein fractions occurs in a liquid medium in a narrow bore capillary (20–200 μm) exposed to extremely high voltages. The velocity of electroendosmosis by positively charged buffer ions from the positive electrode at the inlet towards the negative electrode at the outlet exceeds the electrophoretic mobilities of the protein fractions, leading to a cathodal migration of molecules. These are detected and measured by monitoring absorbance at 214 nm.¹ Results are presented in traditional electrophoretic fractions—albumin, α_1 , α_2 , β , and γ fractions—as percentages of total protein detected. A total protein value, which is measured by Biuret on Abbott Aeroset, is then used in the CZE system to calculate the concentrations of the fractions, including monoclonal bands (in g/litre). However, it had been noted that, in certain samples only, the concentration of albumin calculated by this means gave lower results than albumin measured on the Abbott Aeroset routine analyser by the bromocresol green (BCG) method. Because the BCG albumin result had previously been used in this laboratory as the basis for calculation of monoclonal band concentrations by agarose gel electrophoresis, the introduction of CZE resulted in significant discrepancies in concentrations of certain monoclonal bands compared with previous results. This study aimed to examine differences in albumin estimation between Aeroset BCG, CZE, Aeroset

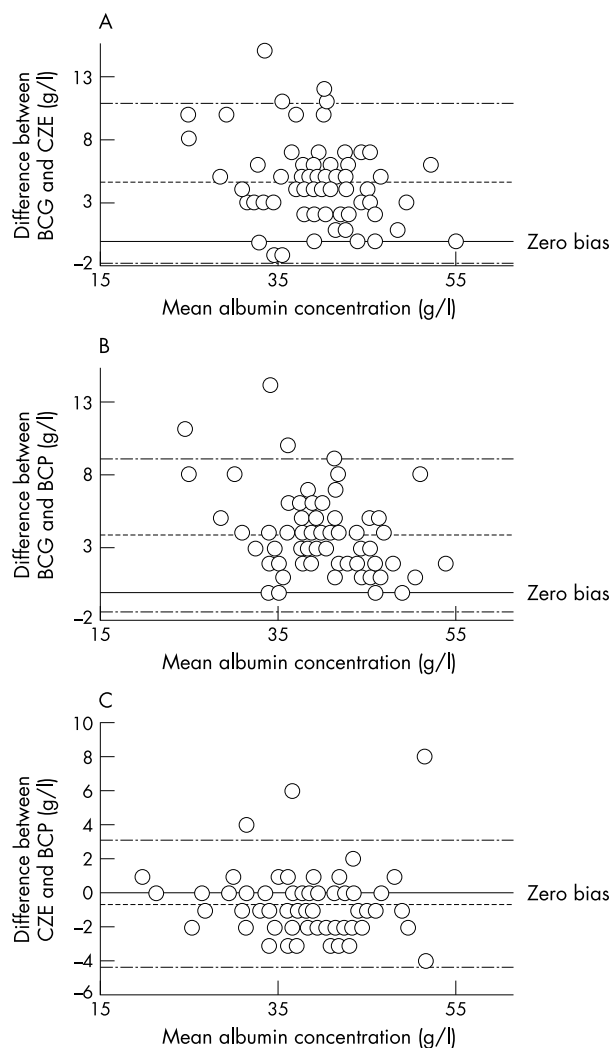


Figure 1 (A) Comparison of albumin measured by Aeroset bromocresol green (BCG) and capillary zone electrophoresis (CZE). (B) Comparison of albumin measured by Aeroset BCG and Aeroset bromocresol purple (BCP). (C) Comparison of albumin measured by CZE and Aeroset BCP.

bromocresol purple (BCP), and the highly specific immunoassay method of albumin measurement.²

Abbreviations: BCG, bromocresol green; BCP, bromocresol purple; CZE, capillary zone electrophoresis

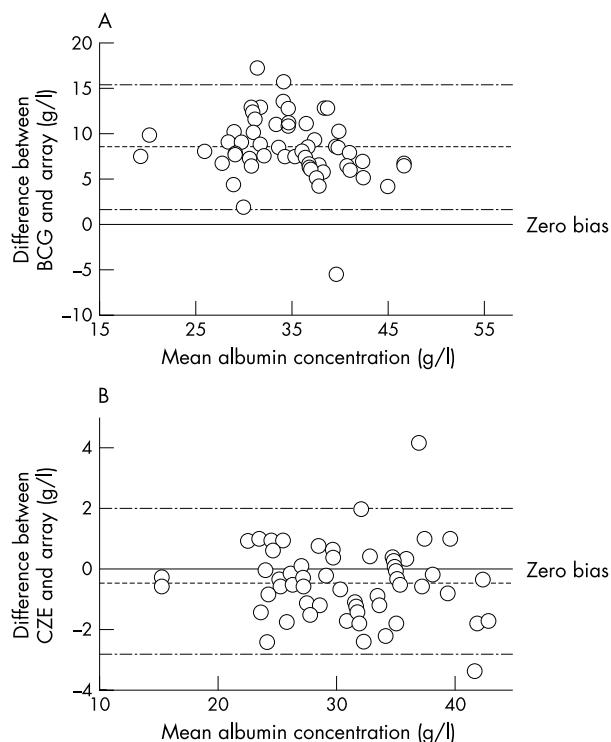


Figure 2 (A) Comparison of albumin measured by Aeroset bromocresol green (BCG) and array immunoassay. (B) Comparison of albumin measured by capillary zone electrophoresis (CZE) and array immunoassay.

METHODS

Albumin was measured on 72 fresh serum samples by Abbott Aeroset BCG (Abbot, Maidenhead, Berkshire, UK; catalogue number, 7D53-01; human based calibrator number, IE65-01), CZE (Beckman Coulter Paragon CZE 2000; Beckman, Fullerton, California, USA), and Abbott Aeroset BCP (catalogue number, 7D54-01). Of these, 42% of BCG results differed by ≥ 5 g/litre from the CZE results. Fifty seven such samples were selected over a three month period and stored at -20°C . Albumin estimations on these were compared between BGG, CZE, and immunoassay (Beckman Array; catalogue number, 449740; human based calibration number, 449730).

RESULTS

Results of Altman and Bland difference plots (fig 1A–C) confirm that Abbott Aeroset BCG results have a positive bias in relation to both CZE and BCP. CZE and BCP agree closely. Figure 2A,B shows that in those samples with discrepancies ≥ 5 g/litre between BCG and CZE, CZE agreed most closely with the gold standard immunoassay.

DISCUSSION

The albumin concentration positively correlates with decreased morbidity and mortality in several diseases and is used in the assessment of patient nutrition and in the calculation of monoclonal band concentrations.^{3 4}

Take home messages

- Bromocresol purple (BCP) is in good agreement with Beckman Array immunoassay, the gold standard for measurement of albumin, and is superior to bromocresol green (BCG), which overestimates serum albumin, particularly in patients with low concentrations of albumin who may also have increased globulins or monoclonal bands
- BCP has replaced BCG as the routine test for albumin in our laboratory

“The results of our study confirm that the Abbott Aeroset bromocresol green method overestimates albumin, possibly because of the long read time”

Bromocresol green binds to albumin at pH 4.2 to form a complex measured spectrophotometrically at 628 nm. However, at low albumin and high globulin concentrations, BCG has been found to bind to α and β globulin fractions. Such binding may be minimised by short read times and by adding succinate buffer and Brij 35 to the reagents.^{5 6} The Abbott BCG method uses succinate buffer, thiomersol, and a read time of approximately 400 seconds. The results of our study confirm that the Abbott Aeroset BCG method overestimates albumin, possibly because of the long read time. This is particularly evident in patients with low concentrations of albumin who may also have increased globulins or monoclonal bands. This effect is not seen with Aeroset BCP, which is now used routinely in this laboratory.

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