

Origins of Dye-Binding Methods for Measuring Serum Albumin

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Featured Article: Doumas BT, Watson WA, Biggs HG. Albumin standards and measurement of serum albumin with bromocresol green. *Clin Chim Acta* 1971;31:87–96.³

Methyl orange and hydroxybenzeneazobenzoic acid (HABA),⁴ introduced in the 1950s, were the first dyes for measuring serum albumin (1). Methyl orange never gained acceptance in clinical laboratories because of its poor specificity, whereas HABA found limited use in the 1960s. In 1967, a trainee from Panama (Osvaldo Hernandez) at the Department of Clinical Pathology of the Medical College of Alabama wanted to present a report at the AACC annual meeting in Philadelphia and asked me (B.T.D.) to give him a “project.” I told him to adapt Bartholomew and Delaney’s bromocresol green (BCG) method for serum albumin (2) to the Technicon AutoAnalyzer. Bartholomew had changed the pH of Rodkey’s BCG reagent from 7.15, at which it had an absorbance of approximately 2.5 absorbance units (3), to a pH of 3.8. There were peaks on the AutoAnalyzer recorder, but repeatability was poor because the AutoAnalyzer coils became covered with a blue sediment—the product of the reaction of BCG with albumin, which was not water-soluble. Addition of the detergent Brij-35 to the BCG reagent (the cure for most problems with AutoAnalyzer methods) turned the color of the BCG reagent to canary yellow and eliminated the blue sediment, but the peaks on the recorder persisted. That was the birth of the BCG albumin method. The presentation at the AACC meeting was received very well by the audience, and the chairman of the session asked, “Is this method really that simple?” Indeed it was.

Following publication of the method and a description of suitable albumin standards in the featured report, laboratories began to abandon the time-consuming and fairly inaccurate “salt fractionation–

biuret” procedure and HABA in favor of the BCG dye-binding method. Within a year, the BCG method was adapted to the Technicon SMA 12/60 multichannel analyzer and to the duPont ACA instrument and became the preferred albumin method. There was no statistically significant difference between albumin values obtained by the BCG method and by cellulose acetate electrophoresis, the gold standard of the 1960s and 1970s. BCG has been documented to be not absolutely specific for albumin, however; it also reacts with α_1 - and α_2 -globulins. With short reaction times (<25 s), the positive bias varies from 1.5 g/L to 5.0 g/L, with a mean value of 3.7 g/L. To improve the specificity, many laboratories opted for the bromocresol purple (BCP) dye (4), which is more specific for albumin than BCG. Today, according to the College of American Pathologists Clinical Chemistry Surveys, 54% of reported results are obtained with BCP methods, and 47% are obtained with BCG methods.

BCG or BCP?

Both methods have advantages and disadvantages, with neither clearly better than the other. BCP methods, although more specific, underestimate albumin in patients undergoing hemodialysis, more so in cases of continuous ambulatory peritoneal dialysis, owing to the presence of 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), an endogenous uremic toxin. This interference is a serious shortcoming; CMPF does not interfere in BCG methods.

Hypoalbuminemia is recognized as an independent risk factor of mortality following renal transplantation. The albumin concentration in patients undergoing hemodialysis is a more powerful predictor of death risk than the urea reduction ratio, and the probability of death is highly associated with a low serum albumin concentration (5). In view of the difference in specificity between BCG and BCP, Blagg et al. recommended, “Dialysis facilities must be aware of the albumin method used by [their] laboratory and, based on the particular method, determine appropriate assurance limits. If serum albumin concentration is to be used as a guide to the probability of mortality or of the occurrence of various complications, whether in dialysis patients or other patients, the specific albumin method used must be taken into consideration” (6). In

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³ This paper has been cited more than 1570 times since publication.

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⁴ Nonstandard abbreviations: HABA, hydroxybenzeneazobenzoic acid; BCG, bromocresol green; BCP, bromocresol purple; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid.

a report on serum albumin to the Network 11 Dialysis Facility Medical Directors, the Medical Review Committee of the Renal Network of the Upper Midwest stated (Dec. 6, 1993), "Dialysis facility personnel should become informed about their lab's albumin assay, calibration standards and reference range. Reference ranges for the serum albumin assay should be based on a normal (e.g., not end stage renal disease or hospitalized patients) population. The goal for adult hemodialysis patients should be to maintain the serum albumin level within the lab's reference range." We have used this information to remind laboratorians and manufacturers that the two methods (BCG and BCP) do not yield the same results for serum albumin (7).

The BCG and BCP methods have dominated the measurement of albumin in the US, and possibly worldwide, for almost 40 years. They are simple, fairly specific, and inexpensive, and most likely will not be retired soon. There is, however, still no primary standard/calibrator for albumin. The development of a Standard Reference Material or a consensus standard is essential for assessing the accuracy of albumin measurements. Equally essential is to establish reference values for both BCP and BCG, because they are not identical.

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