More on Determination of Urinary Albumin by Centrifugal Analysis

To the Editor:

We reply to the criticisms of our procedure for determination of urinary albumin by centrifugal analysis (1). Goeling et al. (2) and Pejacovic and Peake (3) state that 5 mg/L is an appropriate figure for the detection limit. Using our method at both 340 and 440 nm, we assayed 21 replicate samples of a phosphate-buffered saline (PBS) blank and 1.25 mg/L human albumin standard in PBS. We demonstrated a clear difference at both wavelengths between the mean increase in absorbance ± 3 SD of the blank and the mean increase in absorbance − 3 SD of the standard. This difference was significant by the paired-t test (P < 0.001).

In agreement with Bakker (4), we found that the cross reaction of the two bovine serum albumin (BSA) preparations tested (Sigma Chemical Co., products no. A9647 and A4503) with the anti-human albumin antibody (Dako, product no. A001) ranged from 20% to 30% for concentrations from 20 to 100 mg/L. As demonstrated by Pejacovic and Peake (3), the use of 20 or 50 mg of BSA per liter of assay buffer increases the absorbance changes in the standards equivalent to 5 or 15 mg/L, respectively, of human albumin. This may account for the decreased sensitivity reported (2, 3) for the methods involving a BSA-containing buffer.

Standards of human albumin between 1.25 and 80 mg/L prepared in PBS, PBS with 1 g of BSA (product no. A9647) per liter, PBS with 1 g of BSA (product no. A4503) per liter, and PBS with 1 g of gelatin (Sigma, product no. G2625) per liter were assayed in duplicate (Table 1). Standards containing BSA had greater absorbance changes and standards containing gelatin had smaller absorbance changes as compared with the corresponding PBS standard. Gelatin appears to inhibit the antigen–antibody reaction. All standards were re-assayed by an immunoelectrophoretic method (5). The albumin standards that we prepared in PBS compared well (r = 1.0, slope ≤ 0.95) with commercial albumin standards (Behring Diagnostics, Hounslow, Middlesex, U.K.). Results obtained by measurement of peak height agreed with those obtained by the routine immunoturbidimetric method (r = 0.97).

In conclusion: (a) at both 340 nm and 440 nm, 1.25 mg/L is the detection limit in this assay; (b) the use of BSA or gelatin solutions is not a satisfactory means of preventing nonspecific adsorption to assay tubes. Working albumin standards should be prepared by fresh dilution in PBS of a stock standard solution.

References


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