Colorimetric Quantitation of Albumin in Microliter Volumes of Serum

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ABSTRACT

Various non-immunochemical approaches to the quantitation of albumin in serum are reviewed. Salt fractionation techniques are unreliable, with substantial errors in estimating hypoalbuminemic states. Electrophoresis displays biases owing to irregular dye-binding or to densitometric scanning of irregular globulin bands. Currently, the most reliable colorimetric procedure for albumin quantitation is the rapid reaction with brom cresol green. By measuring final absorbance within fifteen seconds of mixing serum with reagent, the interference of globulins is eliminated. A microscale (5 microliter serum) rapid reaction for albumin assay has been developed; it can be readily automated on kinetic or centrifugal analyzers.

Introduction

Albumin is a single chain polypeptide, molecular weight 66,210, with a loop-and-link construction allowing the molecule significant flexibility and stability. Approximately 14 g of albumin are synthesized daily by the liver. Physiologically, albumin functions in the binding and transport of long-chain fatty acid anions, in the binding and detoxification of unconjugated bilirubin and in the transport of thyroid and steroid hormones. Albumin also has significant roles in the binding of circulating drugs and as the major transport agent for metals (Cu++, Mn++, Ni++ and Zn++) in blood. Measurement of serum albumin levels provides an indication of vascular integrity, nutritional status regarding proteins and, to some extent, the functional reserve of the liver.

Recently, it has become evident that many of the analytical procedures for albumin estimation, while giving apparently satisfactory results with normal sera, may be misleading when used to evaluate abnormal sera. Salt fractionation techniques are based on the observation that albumin is more soluble in highly concentrated salt solution, whereas globulins are precipitated; however, sodium sulfate produces neither albumin free globulin or globulin free supernatant. The direction of error in salting-out techniques is most often positive. In pathologic sera, the increase in globulins may result in their incomplete precipitation and a falsely elevated albumin level. Errors are greatest in subjects with cirrhosis, nephrosis or paraproteinemia. Today, salt fractionation techniques are merely of historical importance, and their continued use in
the clinical laboratory should be discouraged.

In its native state, albumin is a highly soluble molecule carrying a strong negative charge of $-19$; it migrates rapidly in electrical fields. In methods using dye-binding following electrophoresis, accuracy depends on the reliability of the total protein estimation. Elevated concentrations of lipoproteins or glycoproteins are not correctly assessed by either the biuret estimation of total protein or by dye-binding following electrophoresis. Binding of Ponceau S is very likely to be non linear if the concentration of globulins exceeds 60 g per liter; any error related to non-linearity will be accentuated in the presence of an increased amount of any single protein fraction. Proteins other than albumin (e.g., esterases) may migrate at the same rate as albumin during cellulose acetate electrophoresis, and densitometric scanning may underestimate irregular globulin bands, leading to over-estimation of albumin.12

Procedures based on dye-binding in solution by spectral shift currently predominate in clinical laboratories.1 Use of 2-(4-hydroxyazobenzene) benzoic acid (HABA), however, is decreasing since blanks are required, and the assay is affected by the presence of hemoglobin, bilirubin, excessive lipoproteins, heparin and various drugs (principally salicylates). Conversely, the use of brom cresol green (BCG) has many advantages.3 BCG has a sensitivity six fold that of HABA, allowing greater dilution of serum to reduce turbidity. The absorbance of the BCG-albumin complex reduces interference by bilirubin and hemoglobin, while the lower pH at which BCG binds to albumin compared with HABA may displace interferences such as heparin, salicylate and bromsulphalein which bind to albumin at physiologic pH.12 BCG is not entirely specific for albumin, since it binds at a slower rate to certain globulins. Recent studies5,6 have shown, however, that accurate and reliable albumin results can be achieved by measuring absorbance immediately following mixing of serum and dye.

**Principle**

When albumin binds to BCG, at 628 nm the dye-albumin color exhibits a substantial difference from that of the free dye, permitting measurement of albumin in the presence of excess BCG. Surfactant (Brij-35) is included in the working dye solution to minimize the absorbance of the reagent blank, to prevent turbidity and to ensure linearity.

**Reagents**

- **Succinate buffer, 0.10 moles per liter, pH 4.0.** Succinic acid (11.9 g) and sodium azide (100 mg) are dissolved in approximately 800 ml of water. The pH is adjusted to 4.2 ± 0.1 with aqueous sodium hydroxide (0.25 moles per liter) and the reagent diluted with water to a final volume of one liter.

- **Brom cresol green stock solution, 0.60 mmoles per liter.** Brom cresol green (419 mg) is dissolved in 10 ml of aqueous sodium hydroxide (0.1 mole per liter) and diluted with water to a final volume of one liter.

- **Working dye solution (0.15 mmoles BCG per liter).** One volume of brom cresol green stock solution is added to three volumes of succinate buffer (0.10 moles per liter). Brij-35, 30 percent, is added (4 ml per liter) and the pH adjusted to 4.2 ± 0.1.

- **Albumin stock standard solution, 100 g per liter.** Pentex liquid human albumin, fraction V.* Solution is stored at 4° C.

- **Albumin working standard solutions, 20 g per litre and 40 g per liter.** Albumin stock standard solution, 100 g per liter, is diluted with aqueous sodium azide solution (0.5 g per liter), and the working standard solutions are stored at 4° C.

* Miles Laboratories, Inc., Kankakee, IL 60901.
Manual Procedure

APPARATUS

The apparatus needed includes a spectrophotometer (628 nm), a micropipetor, (5 µl) and disposable polystyrene cuvets.

PROCEDURE

1. The spectrophotometer is zeroed with working dye solution.
2. Working dye solution (2 ml) is pipeted into each of a series of disposable cuvets.
3. Albumin working standard solution or serum (5 µl) is carefully pipeted onto the wall of the cuvet, above the meniscus of the working dye solution.
4. As expeditiously as possible, the cuvet is capped, mixed by gentle inversion and the absorbance measured. The time elapsed between mixing and measurement of the final absorbance should be no greater than 15 seconds.
5. Lipemic specimens require a serum blank which is prepared by adding 5 µl of sample to 2.0 ml of succinate buffer, 0.10 moles per liter, pH 4.2. The absorbance at 628 nm of the blank, measured with water as reference, is subtracted from the absorbance of the serum sample when mixed with BCG.

CALCULATIONS

The concentration of each serum sample is determined by comparing its absorbance to those of the two standards used.

Automated Procedure

APPARATUS

The apparatus needed is System 3500 Analyzer with a preprogrammed magnetic card.*

INSTRUMENT SETTINGS

The settings for the instrument are (A) wavelength—628 nm; (B) temperature of 25° C; (C) dispenser tower-setting in position 2; (D) sample syringe stop at 10 µl and (E) dispenser A syringe stop at 2.0 ml.

PROCEDURE

The timing sequence for serum albumin assay by the rapid BCG procedure is shown in table I. The procedure has a productivity of 161 assays per hr and is linear through 60 g per liter. Twenty sequential assays of a human serum pool (39 g of albumin per liter) resulted in a coefficient of variation of 0.56 percent. Eighty serum samples, chosen without conscious bias from the hospital population, were assayed by the automated, rapid BCG procedure and also by the SMA 12/60 BCG methodology†. The good correlation between the two methods (r = 0.984) is shown in figure 1. However, the slope of the regression line would indicate that the SMA 12/60 method overestimates very low levels of albumin, while slightly underestimating high levels.

Discussion

Webster13 originally demonstrated that BCG is not totally specific for albumin, reacting also with α- and β-globulins, but not with γ-globulins. Subsequently, Gustafsson5 showed that the BCG reaction with protein occurred in two phases: an immediate reaction that was specific for

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* Gilford Instrument Laboratories, Inc., Oberlin, OH 44074.
† Technicon Instruments Corporation, Tarrytown, NY 10591.
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Figure 1. Correlation between rapid albumin assay (Gilford System 3500 Analyzer) and Technicon SMA 12/60 BCG assay.

albumin, followed by a slower reaction with the serum constituents (α₂-globulin, orosomucoid, ceruloplasmin) that are elevated in the "acute phase reaction" following infection or tissue injury. More recently, King et al. have demonstrated by stopped flow studies that the albumin-BCG reaction is complete within six seconds after mixing and that other serum proteins begin to interfere at 15 seconds. Thus, accurate albumin quantitation depends on strict timing and expeditious performance of the dye-binding assay. Webster has shown that, using the standard 10 minute incubation period, albumin in normal sera is over-estimated by an average of eight percent. In hypoalbuminemic sera, the additive error ranges from 12 to 30 percent.

Sources of Error

Standardization of albumin assays depends on the availability of a reliable protein standard. At present, human serum albumin fraction V is the most acceptable material for the preparation of albumin standards. It cannot, however, be used interchangeably with bovine preparations since the latter have less dye-binding ability. Liquid preparations of human serum albumin fraction V are preferred, since the powdered form may yield lower serum albumin levels.

Reference Interval (Adults)

The reference interval for adults is 35 to 50 g per liter.

Résumé of Clinical Interpretations

Hyperalbuminemia is found only in the presence of dehydration. The commonest cause of hypoalbuminemia is non-specific: almost every pathologic state is associated with a relative or absolute decrease in serum albumin levels. Lowest levels (less than 20 g per liter) of serum albumin occur with
nephrotic syndrome and protein-losing enteropathies. Hypoalbuminemia is a reliable index of severity and prognosis in chronic hepatic disease (cirrhosis); in acute hepatic disease (viral or toxic hepatitis), serum albumin levels are usually normal or only mildly depressed.\(^8\) A negative nitrogen balance may be responsible for the hypoalbuminemia of major infections, surgical and accidental trauma, eclampsia, uremia, gastrointestinal diseases and myocardial infarction. Malabsorption and malnutrition may cause low albumin levels because of amino acid deficiency. Hypoalbuminemia is also observed in extensive burns and myeloma and macroglobulinemia.

In pregnancy, albumin levels are approximately 14 percent below normal, while oral contraceptive use diminishes albumin levels by approximately 7 percent;\(^{10}\) however, normalization of albumin levels occurs postpartum or after discontinuance of oral contraceptives.

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References