ALBUMIN (BCG)
MANUAL/AUTOMATED APPLICATIONS
C244-01, V244-12

Intended Use
For **IN VITRO quantitative** determination of Albumin in serum or plasma using manual or automated applications.

Clinical Significance
Measurements of Albumin are used primarily for diagnosing liver and kidney diseases, as well as for monitoring the causes and treatments. (1)

Method History
Rodkey (2) in 1965 reported the use of 3,3,5,5 tetrabromo-m-cresolphthalein (BCG) as a specific Albumin binding dye for the quantitation of Albumin in serum. Doumas, et al (3) optimized the BCG-reaction pH for better specificity and adjusted the concentration of polyoxyethylene-23-lauryl ether (Brij-35) to maximize linearity, prevent turbidity and to reduce the absorbance of the reagent baseline at 630nm. Catachem's BCG Albumin method is based upon the work of Doumas, et al.

Method Principle
BCG dye at a pH of 4.2 and in the presence of non-ionic surfactants including Brij-35, specifically binds Albumin to form a colored BCG-Albumin complex with maximum absorbance at 630nm. The increase in absorbance is directly proportional to the Albumin concentration present in the sample.

pH 4.2
Albumin+BCG----------> BCG-Albumin complex

Reagent Content
The concentrations of the active ingredients in the reagents are approximately as follows.

BCG Reagent
Each liter contains:

Water	
BCG	0.32 mmol/L
Buffer	
Nonreactive ingredients and stabilizers	

Precautions
Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation of Working Reagents
Catachem's BCG Reagent is in ready-to-use form**.**

Reagent Storage and Stability
Store the Albumin Reagent at 25˚C. When stored as directed, this reagent is stable until the expiration date stated on the label.

Specimen Collection and Preparation
Test sera should be fresh, clear and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay.

Interfering Substances
Slight or moderate lipemic serum, containing more than 800 mg/dL Triglycerides could increase the Albumin values by 0.2 g/dL or greater. Samples with Bilirubin content of 30 mg/dL do not significantly affect the Albumin values. Samples with Hemoglobin content of 300 mg/dL or less do not affect the Albumin results. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (4)

Expected Values
Normal serum from a healthy human adult contains about 3.5 to 5.0 g of Albumin/dL, averaging about 4.5. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the species under evaluation and for the area in which the laboratory is located.

Procedure
Important: Read the entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	Appropriate to dispense 1 ml
Pipette	Appropriate to dispense 8 µl
Constant temperature	incubator for assays at 37˚C

Materials Provided

Albumin BCG Reagent.

353 Christian Street, Suite #2, Oxford, Connecticut 06478 USA ● Phone (203) 262-0330 ● Fax (203) 262-9836 ● email: catachem@catacheminc.com

Analytical Parameters

Wavelength	630nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	60 seconds
Reaction Volume	1.0 ml
Sample Volume	0.008 ml
Total Volume	1.008 ml
Sample-to-reagent ratio	1:126

Assay Procedure

1. Pipette 1.0 ml of BCG reagent into each of three cuvettes marked “Calibrator”, “Sample”, and “Blank”.
2. Pipette 0.008 ml of sample(s) and calibrator into their respective cuvettes. Use 0.008 ml water for the blank. Mix all cuvettes well.
3. Incubate all cuvettes for 1.0 minute at 37˚C.
4. Set spectrophotometer wavelength at 630nm and zero the instrument with the cuvette marked “blank”.
5. Read the “Calibrator” and “Sample” absorbencies.
6. Calculate the Albumin concentration (g/dL) in the sample(s), as shown in results and calculations.

Results and Calculations

$$\text{ALB (g/dL)} = \frac{\text{Sample Absorbance}}{\text{Calibrator Absorbance}} \times \text{calibrator (g/dL)}$$

Example:

sample absorbance	=	0.300
calibrator	=	0.250
calibrator value	=	4.0 (g/dL)

$$\text{ALB (g/dL)} = \frac{0.300}{0.250} \times 4.0 = 4.8 \text{ g/dL}$$

Quality Control

To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum.

Method Performance Characteristics

Sensitivity: The sensitivity of this method is 0.140-0.180 absorbance units per g/dL.

Linear Range: In this method there is no significant nonlinearity over the range of 0-6 g/dL.

Precision: Within-run and day-to-day precision is summarized below.

Precision Study

ALBUMIN	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
g/dl	g/dL	%	g/dL	%
1.2	0.04	3.5	0.05	4.3
3.4	0.05	1.4	0.07	2.0
5.6	0.06	1.1	0.06	1.0

Correlation

A comparison of this method using an automated analyzer and a reference method based upon the BCG reaction resulted in the following regression statistics.

Range	=	1.6 - 5.2 g/dL
N	=	197
Y	=	1.039 - 0.12
r	=	0.999
Sy.x	=	0.09

References

1. Fundamentals of Clinical Chemistry edited by Norbert Tietz. WB Saunders, Philadelphia (1976).
2. Rodkey FL. Direct spectrophotometric determination of albumin in human serum. Clin Chem 11 (1965).
3. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurements of serum albumin with bromocresol green. Clin Chem Acta 31, 87-96 (1971).
4. Young DS, Pestaner LC, Gibberman V. Effects of drugs on clinical laboratory tests. Clin Chem 21, No 5 (1975).

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