POINTE SCIENTIFIC, INC.
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# Albumin Reagent Set

## Intended Use
For the quantitative determination of Albumin in serum. For *in vitro* diagnostic use only.

## Method History
Determination of serum albumin is usually made using an ultra centrifugation, salt fractionation, electrophoretic or dye binding method. Dye binding procedures are the simplest to perform, and lend themselves to high volume testing and automation. They are also the procedures most widely used in combination with total protein determinations to yield an A/G ratio.[1,2] In 1953, the use of methyl orange[3] for direct determination was described. This method suffered from non-specific binding characteristics.[4,5] The use of a HABA[6] dye was introduced in 1954. This method was specific for albumin but displayed poor sensitivity, poor correlation with electrophoresis methods and significant interference from bilirubin, lipids, salicylates, penicillin and sulfonamides.[7]

A bromocresol green (BCG) dye-binding procedure was first proposed in 1964.[8] This procedure exhibited greater sensitivity and much lower susceptibility to interfering substances. The original method has been optimized to improve correlation with electrophoretic methods.[9] The present procedure follows a modification of the original BCG dye-binding procedure.

Several publications of the late 1970's [10,11,12,13] reported that abnormal proteins will bind with BCG after the first minute. The present procedures include a reduced measuring time to eliminate abnormal globulin interference and offers linearity to 8.0 g/dl.

## Principle
Albumin is bound by the BCG dye to procedure an increase in the blue-green color measured at 600/700 nm. The color increase is proportional to the concentration of albumin present.

## Reagents
Bromocresol Green (BCG) 0.15 g/L, Buffer, pH 4.66± 0.1, surfactant, non-reactive ingredients and stabilizers.

## Reagent Preparation
Reagent is in a "ready to use" state.

## Reagent Storage
Store reagent at room temperature.

## Reagent Deterioration
The reagent should be clear, yellow-green solution. Turbidity or precipitation makes the reagent unsatisfactory and it should be discarded.

## Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Avoid ingestion.
3. Avoid contact. Reagent is an acid solution. Flush with water when contact occurs.
4. Reagent contains Sodium Azide as a preservative. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

## Specimen Collection and Storage [14]
1. Serum is the specimen of choice.
2. Avoid excessive hemolysis since every 100 mg/dl of hemoglobin corresponds to about 100 mg/dl of albumin.
3. Albumin in serum is reported stable for one week at room temperature (18-30°C) and approximately one month when stored in the refrigerator (2-8°C) and protected against evaporation.

## Interferences
1. See Young et al[15] for a list of interfering substances.
2. Ampicillin has been found to seriously interfere with BCG methods.[16]

## Materials Provided
Albumin reagent.

## Materials Required but not Provided
1. Beckman Coulter AU™ analyzer.
2. Chemistry Controls
3. Chemistry Calibrator.
4. Instrument application and Operation manual.

## Procedure (Beckman Coulter AU™400 application)

**SPECIFIC TEST PARAMETERS**

TEST NUMBER: **#** TEST NAME: **Albumin** ∇ TYPE: **Serum** ∇ OPERATIONAL: **Yes** ∇
SAMPLE VOL.: **2** DIL. VOL.**: 0** PRE-DILUTION RATE: **1**
REAGENTS: R1 VOLUME: **200** DIL. VOL.**: 0** MIN. OD MAX. OD
R2 VOLUME: **0** DIL. VOL.**: 0** L H
REAGENT OD LIMIT:
WAVELENGTH: PRI. **600** ∇ SEC. **800** ∇ FIRST L: **0.0000** FIRST H: **0.7500**
METHOD: **END** ∇ LAST L: **0.0000** LAST H: **0.7500**
REACTION SLOPE: **+** ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: **0** LAST: **1** L: **#** H: **#**
MEASURING POINT 2: FIRST: LAST: CORRELATION FACTOR:
LINEARITY: % A: **1.000** B: **0.000**
NO LAG TIME: ∇ ON BOARD STABILITY PERIOD: **#**

**SPECIFIC TEST PARAMETERS**

VALUE FLAG: **#** ∇ LEVEL L: LEVEL H:

| NORMAL RANGES: | SEX | AGE L YEAR | AGE L MONTH | AGE H YEAR | AGE H MONTH | L | H |
|---|---|---|---|---|---|---|---|
| ○ 1. | # ∇ | # | # | # | # | # | # |
| ○ 2. | # ∇ | # | # | # | # | # | # |
| ○ 3. | # ∇ | # | # | # | # | # | # |
| ○ 4. | # ∇ | # | # | # | # | # | # |
| ○ 5. | # ∇ | # | # | # | # | # | # |
| ○ 6. | # ∇ | # | # | # | # | # | # |
| 7. NONE SELECTED | | | | | | # | # |
| 8. OUT OF RANGE | | L | | H | | # | # |

PANIC VALUE: **#** **#** UNIT: **g/dl** DECIMAL PLACES: **1**

**CALIBRATION SPECIFIC PARAMETERS**

CAL TYPE: **AB** ∇ FORMULA: **Y=AX+B** ∇ COUNTS: **2** PROCESS: **CONC.** ∇

| | CAL. NO. | OD | CONC. | FAC/OD-L | FAC/OD-H |
|---|---|---|---|---|---|
| POINT 1. | # | | # | **-9999999** | **9999999** |
| POINT 2. | | | | | |
| POINT 3. | | | | | |

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| | | |
|---|---|---|
| POINT 4. | | |
| POINT 5. | | |
| POINT 6. | | |
| POINT 7. | | |
| 1-POINT CAL. POINT: | ○ | WITH CONC-0 |
| MB TYPE FACTOR: | | CALIBRATION STABILITY PERIOD: # |

**#: User-Defined**

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with your laboratory Quality Control Program for validation.

**NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853**

## Limitations

1. The dye-binding properties of albumin, other than human, differ among species.[17]
2. Samples with values above 8.0 g/dl should be diluted with 0.9% saline 1:1, re-run, and results multiplied by 2. Samples with results below 0.5 g/dl should be done electrophoretically.

## Calibration

The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be recalibrated.

## Calculation

Abs. = Absorbance

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Conc. Of Std.} = \text{Albumin (g/dl)}$$

Example: If the Absorbance of the Unknown = 0.200 and the Absorbance of the Standard is 0.19 and the Standard Concentration = 3.5, then:

$$\frac{0.200}{0.190} \times 3.5 = 3.68 \text{ g/dl}$$

## Quality Control

The validity of the reaction should be monitored by use of normal and abnormal control sera with known albumin concentrations.

## Expected Values[1]

3.5 – 5.3 g/dl

It is strongly recommended that each laboratory establish its own normal range.

## Performance

1. Linearity: 0.5 – 8.0 g/dl
2. Comparison: A comparison study performed between a Roche Hitachi 717 and Beckman AU400 using this BCG method resulted in a correlation coefficient of 0.974 with a regression equation of y = 0.94x-0.35.
3. Precision:
   Within - day precision study was performed using three levels of material.
   Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.

| Within Day (N=20) | | | Day to Day | | |
|---|---|---|---|---|---|
| **Mean** | **S.D.** | **C.V.%** | **Mean** | **S.D.** | **C.V.%** |
| 2.6 | 0.07 | 2.7 | 2.0 | 0.04 | 2.0 |
| 4.8 | 0.05 | 1.0 | 4.0 | 0.13 | 3.3 |
| 5.7 | 0.06 | 1.1 | | | |

Precision and Linearity studies were performed following modifications of CLSI Protocols EP5 and EP6[18] using a Beckman Coulter AU™400 analyzer

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