

Albumin

1.0 INTENDED USE

This reagent is intended for the quantitative determination of albumin in serum.

2.0 BACKGROUND

2.1 METHOD AND HISTORY

Methodologies for the determination of serum albumin and globulin include electrophoresis, salt fractionation, glyoxylic acid-tryptophan reaction, and dye binding techniques (10.1-10.3). The electrophoresis and salt fractionation methodologies are too involved for routine or stat work.

The bromocresol green method for determination of serum albumin is the most specific and sensitive of the dye binding techniques (10.4). The glyoxylic acid method measures tryptophan content which represents 8-10% albumin and 90-91% globulin. Since the bromocresol green method is specific and simple, it is the method of choice for albumin determination.

2.2 TEST PRINCIPLE

Serum albumin reacts with bromocresol green to form a colored complex. The color is proportional to the amount of albumin present.

2.3 CLINICAL SIGNIFICANCE

Elevated levels of albumin are often indicative of dehydration, rather than the presence of a pathological condition. Low albumin levels are often associated with edema, and are also found in nephrotic conditions due to loss of albumin into the urine.

Serum albumin is synthesized in the liver. Hepatic disorders, such as cirrhosis and hepatitis, interfere with protein synthesis and will lower the level of albumin in circulation.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. Hemolyzed samples should not be used.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25 μ l. Call Biotron's technical service department at 1-800-595-8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

In the absence of bacterial contamination, serum albumin levels remain unchanged after 4 days at room temperature (18°-26° C.) 2 weeks refrigerated (2°-8° C.) or for 6 months frozen (-20° C.) Frozen samples should be thawed at room temperature and mixed thoroughly before analysis. Thawed samples should not be refrozen. (10.5)

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur within 4 hours of collection, store the sample properly using the guidelines above.

4.0 MATERIALS

(2 X 125 ml)

(1 X 500 ml)

Reagents necessary for the determination of albumin are included in the kit.

4.1 ALBUMIN REAGENT

Albumin reagent contains:

bromocresol green sodium salt

0.21 mM

sodium citric buffer

104 mM

preservatives and stabilizers

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

4.3 REAGENT PREPARATION

The reagent is ready to use as is.

4.4 REAGENT STORAGE AND STABILITY

When stored at room temperature (18-26° C) the reagents are stable until the expiration date stated on the labels.

4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 630 nm.

4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 630 nm.

4.5.3 Test tubes capable of holding 3 ml.

4.5.4 Pipettes capable of delivering 2 ml and 10 μ l.

4.5.5 Timer for a 1 minute incubation.

4.5.6 Calibrator

4.5.7 Normal and abnormal control for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength

630 nm

Temperature

18-26° C or 37° C

Pathlength

1.0 cm

Mode

endpoint

Reaction time

1 minute

Sample volume

10 μ l

Reagent volume

2.0 ml

Total volume

2.01 ml

Sample to reagent ratio

1/200

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 630 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

5.4 The albumin assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

5.4 PROCEDURE

5.4.1 Into separate test tubes pipette 10 μ l of distilled water, calibrator, or serum to be assayed.

5.4.2 Add 2.0 ml of albumin reagent and mix.

5.4.3 Incubate at 18-26° C (room temperature) for 1 minute.

5.4.4 Determine the absorbance of the calibrator (A_s) and of each unknown (A) at 630 nm using the distilled water as the reagent blank.

5.5 PROCEDURE NOTE

The color of the final reaction mixture is stable for 1 hour.

5.6 CALCULATION AND RESULTS

$$\text{albumin (g/dl)} = \frac{A}{A_s} \times \text{albumin value of calibrator}$$

A = absorbance of sample

A_s = absorbance of calibrator

Example:

$$\text{albumin concentration} = \frac{.650}{.540} \times 4.0 \text{ g/dl} = 4.8 \text{ g/dl}$$

with A = .650, A_s = .540, albumin value of calibrator = 4.0 g/dl

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.5)

The range of expected values is: 3.2 - 5.3 g/dl

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 MEDICAL ALERT VALUES (10.7)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.3 LIMITATIONS OF PROCEDURE

A comprehensive list of drugs and other substances which can affect the albumin concentration in serum is given by Young. (10.6)

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

The albumin assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details.

Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

In this study, 15 replicates of 2 control sera were run.

<u>Mean (g/dl)</u>	<u>SD (g/dl)</u>	<u>CV (%)</u>
2.19	± 0.02	1.14
3.73	± 0.09	2.29

Between-Run

In this study, 5 runs were made, each run consisting of 5 replicates of 2 control sera.

<u>Mean (g/dl)</u>	<u>SD (g/dl)</u>	<u>CV (%)</u>
2.96	± 0.11	3.58
3.49	± 0.16	2.96

9.2 CORRELATION

A correlation study was done on the Technicon RA-500 system at 37° C comparing this method and a similar albumin (BCG) method. The samples range between 0.3 g/dl and 5.8 g/dl.

<u>Number of Samples</u>	<u>Regression Equation</u>	<u>Correlation Coefficient</u>
48	$y = .905x + .169$	0.997

9.3 LINEARITY

This procedure is linear through 8 g/dl beyond which the specimen should be diluted with an equal volume of deionized water. Reassay the specimen and multiply the results by 2.

10.0 REFERENCES

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- 10.6 Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., Washington DC, AACC Press (1990).
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