ALBUMIN

INTENDED USE

Vitro albumin reagent is intended for the in vitro quantitative determination of albumin in serum or plasma on both automated and manual systems.

METHOD

Colorimetric endpoint method according to modified bromcresol green binding assay (BCG) (Doumas et al)

CLINICAL SIGNIFICANCE

Albumin is a carbohydrate-free protein, representing 55 – 65% of the total plasma proteins. It is synthesized in the liver and is noted for its ability of configuration changes. It maintains the plasma colloidal osmotic pressure, transports and stores a wide variety of ligands and serves as a source of endogenous amino acids. Albumin binds and solubilizes a variety of compounds amongst which are bilirubin, calcium, and long-chain fatty acids. Albumin also binds toxic heavy metal ions and many drugs, which is why a decrease in albumin in the blood can have important pharmacokinetic consequences[2]. Hyperalbuminemia is of little diagnostic significance except in dehydration. Hypoalbuminemia is very common in many diseases and stems from various factors: impaired synthesis, either primary as a result of liver disease or secondary due to diminished protein intake; increased catabolism because of tissue damage (severe burns) or inflammation; malabsorption of amino acids (Crohn's disease); proteinuria due to nephrotic syndrome; protein loss by way of feces (neoplastic disease). In severe hypoalbuminemia plasma albumin levels are below 2.5 g/dl. The low plasma oncotic pressure allows water to move out of the blood capillaries into the tissues (edema). Albumin measurements also allow monitoring of the patient's response to nutritional support and are a useful test of liver function[3].

ASSAY PRINCIPLE

For a number of years the standard method for albumin determination was measurement of protein remaining in solution following salt precipitation of globulin fractions[4]. Electrophoresis has also been widely used. Measurement of albumin has been greatly simplified by the introduction of dye binding methods[5, 6]. The method using bromcresol green (BCG)[7] is more specific, sensitive, and less prone to pigment interference than the earlier dye binding methods, and has been improved by reduction of the reaction pH.

Albumin at pH 4.2 is sufficiently cationic to bind the anionic dye bromcresol green (BCG) to form a blue-green colored complex.

Albumin + BCG $\xrightarrow{pH\ 4.2}$ **Albumin-BCG complex**

The intensity of the blue-green color is directly proportional to albumin concentration in the specimen. It is determined by measuring the increase in absorbance at 580 - 630 nm.

EXPECTED VALUES

Adults[8]	
18 – 60 years	3.5 – 5.0 g/dl
> 60 years	3.4 – 4.8 g/dl
Children	
14 – 18 years	3.2 – 4.5 g/dl
4 days - 14 years	3.8 – 5.4 g/dl
Newborns	
< 4 days	2.8 – 4.4 g/dl

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the albumin results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

R_1	Albumin standard	4.0	g/dl
R_2	Succinate buffer, pH 4.2	75.0	mmol/l
	Bromcresol green	0.26	mmol/l

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

SPECIMENE

Serum or plasma*

* The only accepted anticoagulants are heparin and EDTA.

Specimen Preparation & Stability For serum specimen

Patients should be following their usual diet and be in their usual state of health. Serum should be separated immediately from the clot. Analyze specimen promptly or store for less than 3 days at 4^{0}C. Albumin in the specimen remains stable for 6 months when frozen at –20^{0}C or indefinitely at -70^{0}C[9].

PROCEDURE

- **Manual Procedure**

Wavelength	580 - 630 nm
Cuvette	1 cm light path
Temperature	20 - 25 ^{0}C
Zero adjustment	against reagent blank
Specimen	Serum or plasma

	Blank	Standard	Specimen
R_2	2.0 ml	2.0 ml	2.0 ml
Standard	……	10 µl	……
Specimen	……	……	10 µl

Mix, and Incubate for 5 minutes at room temperature Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.

The color is stable for 30 minutes.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request

CALCULATION

Calculate the albumin concentration in serum by using the following formulae:

Serum albumin Concentration=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard}$$

- **Unit conversion**

g/dl x 1.45 = µmol/l

Expression of results in µmol/l is only used for binding albumin.

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QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established albumin values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  Heparin and EDTA are the only accepted anticoagulants.
- **Bilirubin:**
  No significant interference from free or conjugated bilirubin up to a level of 60 mg/dl.
- **Drugs:**
  Young[9] in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.
- **Haemoglobin:**
  Haemoglobin levels higher than 3.0 g/l increase the apparent albumin concentration significantly.
- **Lipemia:**
  Intralipid levels higher than 1.0 g/dl increase the apparent albumin concentration significantly.

WARNING & PRECAUTION

Vitro albumin reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.

- The reagents should be brought to room temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- Don't use the reagent if it is turbid.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run		Between Day	
Control	Level I	Level I	Level II	Level II
Number of samples	40	40	40	40
Mean (g/dl)	2.8	2.8	4.4	4.4
SD (g/dl)	0.47	0.7	1	0.6
CV (%)	1.7	2.2	2.4	1.4

Method Comparison

Comparison studies were carried out using another commercially available BCG method for Albumin as a reference. Normal and abnormal human serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	45
Range of results	0.7 - 4.8 g/dl
Slope	0.935
Intercept	0.17 g/dl
Correlation coefficient	0.979

Sensitivity

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a pathlength of 1 cm.

When run as recommended the sensitivity of this assay is 0.2 g/dl.

LINEARITY

When run as recommended, the assay is linear up to 7.0 g/dl.

If result exceeds 7.0 mg/dl, specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE	REF	SIZE
10101	2 X 100 ml	10105	4 x 250 ml
10102	2 X 125 ml	10106	2 x 500 ml
10103	4 X 125 ml	10107	1 x 1000 ml
10104	1 X 500 ml		

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