ALBUMIN BCG (BROMOCRESOL GREEN)

Diagnostic reagent for determination of Albumin concentration.
Liquid. Monoreagent. Store at +15/+25°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Pack
MH-001	160 mL
MH-002	60 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of Albumin in serum and plasma.

GENERAL INFORMATION

The name albumin (L. albus, meaning white) stems from the white precipitate formed during the boiling of acidic urine from patients with proteinuria. Normally, albumin is the most abundant plasma protein from the fetal period onwards and makes up about half of the plasma protein mass. It is a major component of most body fluids, including interstitial fluid, cerebrospinal fluid (CSF), urine and amniotic fluid. More than half of the total albumin pool is in the extravascular space.[2] A very small amount is lost from the body through excretion. It is catabolized in various tissues and taken up by cells through pinocytosis. Its constituent amino acids are released by intracellular proteolysis and returned to the body pool.[4]

Albumin has an unglycosylated polypeptide chain of 585 amino acids and a molecular weight of 66,438 Da. Albumin is synthesized by hepatic parenchymal cells in response to colloidal osmotic pressure and dietary protein intake[1] and has a 3D structure stabilized by 17 intra-chain disulfide bonds.[2,3]

The rate of albumin synthesis is also subject to feedback regulation determined by plasma albumin concentration.[4] It is both chemically and biologically stable because it is resistant to denaturation at higher temperatures than most plasma proteins and remains in circulation with a half-life of 15 to 19 days. Albumin contains a large amount of charged amino acids, which contribute to high solubility, and has a net negative charge of approximately 212 at neutral pH. Therefore, albumin contributes approximately 6 to 10 mmol/L to the anion gap at normal albumin concentrations of 0.5 to 0.8 mmol/L (3.5 to 5.2 g/dL) and less at lower albumin concentrations.[2,5]

Albumin has two critical biological functions: First, it acts as the main component of colloid osmotic pressure.[2] It constitutes 75% of the colloid osmotic pressure of plasma. When albumin levels drop significantly to about 20 g/L (e.g. nephrotic syndrome), edema is often seen due to decreased colloid osmotic pressure. Decreases in serum albumin can occur in response to many pathologic events, so changes in serum albumin are not very specific.[2,4] The latter serves as a carrier for a wide variety of substances, including fatty acids and other lipids, bilirubin, foreign substances such as drugs, thiol-containing amino acids, tryptophan, calcium and metals. Some of these substances, such as fatty acids and unconjugated bilirubin, have very low solubility in water in the absence of a carrier molecule.[2] Albumin also binds many hormones such as thyroxine, triiodothyronine, cortisol and aldosterone. It thus acts as a reservoir in which these physiologically potent compounds are stored in an active form but from which they can be readily mobilized. This binding property extends to xenobiotic compounds such as drugs. Albumin binds salicylate, valproate, phenytoin, warfarin, phenylbutazone, clofibrate and many other drugs. Therefore, low albumin values may explain why drug toxicity develops when the concentration of the drug in serum is measured, even though they are at apparently low concentrations. In addition to its role as a molecule of binding and transport, albumin also plays a major role in the nutritional status. It is suggested that the protein is structured in such a way that it is easily metabolized and contains all the necessary amino acids.

In starvation, the plasma concentration of albumin decreases significantly more than gamma globulin levels. Very low concentrations are observed in malnutrition, especially in kwashiorkor (diets deficient in protein and calories).[4]

Albumin is a negative acute phase reactant and tends to have low concentrations in most patients in intensive care units.[6]
The permeability of capillaries increases, which causes albumin to leak into the extracellular space in inflammation. Since many macronutrients are bound to albumin and other circulating proteins, there may be transient decreases in the measured concentration.[7]

Although plasma albumin measurement is important for management and control, it is still considered less valuable for clinical diagnosis. Hyperalbuminemia is usually due to dehydration or hemoconcentration. Hypoalbuminemia is the result of (1) hemodilution; (2) a synthesis rate lower than the rate of albumin depletion; (3) diseases that cause a large loss of albumin from the urine, skin or intestine; or (4) increased catabolism, as in fever, untreated diabetes and hyperthyroidism. Decreased synthesis may be due to the inability of the liver to synthesize albumin as a result of malnutrition, malabsorption or diseases such as acute or chronic hepatitis. Low plasma albumin concentration may

be the result of large losses in diseases such as nephrotic syndrome, protein-losing enteropathy, exudative skin lesions or burns. Burns in particular may be associated with severe albumin loss.[4]

In chronic liver disease, albumin is a good indicator of prognosis and has been used in the Child-Pugh scoring system.[9] Currently, the MELD (model for end-stage liver disease) score[10] is widely used and does not include albumin. Some objections to this have recently been raised by Brown et al. It has been argued that the Child-Pugh score is a better predictor of survival than the MELD score in certain situations because albumin level is the most useful predictor of survival.[4,11] There are more than 20 inherited variants of albumin which are not associated with disease. This includes bisalbuminemias in which two chemical types of albumin are present. Congenital absence of albumin, or analbuminemia, is asymptomatic except for occasional slight edema.[4]

TEST PRINCIPLE

Colorimetric measurement

In citrate buffer, albumin forms a colored component with green bromocresol (BCG) and the color intensity is proportional to the albumin concentration. More specific results are obtained if the reaction can be timed and readings taken shortly after mixing of the sample and reagent. The color intensity (blue-green color) measured at a wavelength of 630 nm is proportional to the albumin concentration in the sample.

REAGENT COMPONENTS

Bromcresol Green : ≤ 0.3 mmol/L
Succinic acid : ≤200 mmol/L
Sodium azide :<%0.1
Surface active agent

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +15/+25˚C till the expiration date stated on the label which is only for closed vials.
Once opened vials are stable for 45 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[12]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. For plasma, sample collection tubes with Li heparin should be preferred. Multiple sample freezing and thawing should be avoided.

Albumin activity stability in serum and plasma:[28]

2.5 months at +20/+25ºC
5 months at +2/+8ºC
4 months at -20°C

Annotation:

- Heparin in blood tubes has been reported to interfere with some dye binding methods.[13] However, it has been noted that heparinized plasma can also be used if precautions are taken to avoid heparin interactions.[14]
- Fasting is not necessary, although desirable, as only in the presence of significant lipemia is the BCG test affected.[4]
- Venostasis should be avoided when collecting samples; hemoconcentration increases the apparent concentrations of albumin and other plasma proteins.[4]

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of Arcal Auto Calibrator.

Arcal Auto Calibrator (Lyophilized)
Ref.No: VT-003

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control (Lyophilized)
Ref.No: VT-001

Arcon P Level 2 Control (Lyophilized)
Ref.No: VT-002

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Expected Values [8]

Serum/Plasma

Age	Range (g/dL)
0 day to 4 days	2.8 – 4.4
4 days ile 14 years	3.8 – 5.4
14 years to 18 years	3.2 – 4.5
18 years to 60 years	3.5 – 5.2
60 years to 90 years	3.2 – 4.6
>90 years	2.9 – 4.5

Annotation:

- According to the IFCC/BCR/CAP reference material CRM 470, the current provisional consensus reference range for albumin in serum is 3.5 to 5.2 g/dL for healthy white adults and adolescents.[15]

Albumin concentrations reach these levels around 20 to 30 weeks of pregnancy and remain relatively constant until at least 20 years of age, after which they gradually decrease with age. However, concentrations are significantly lower in premature infants.[16]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[17]

Unit Conversion:
g/dL x 10 = g/L

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[18]
The determined analytic measuring interval for Albumin is 0.2 – 8 g/dL.

Detection Capability

Limit of Detection (LoD): 0.1 g/dL

Limit of Quantitation (LoQ): 0.2 g/dL
Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[19]

Linearity

This method shows measurement linearity in the activities up to 8 g/dL. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[20]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[21]

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Albumin have been given in the table 1 and 2 respectively.

Table 1. Albumin Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
2.38 g/dL	0.03	1.38	80
4.54 g/dL	0.05	1.20	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[22]

Table 2. Albumin Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
2.38 g/dL	0.05	2.04	80
4.54 g/dL	0.10	2.28	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[22]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:[23]
y= 1.009x – 0.195
r= 0.99

Interference

Endogenous interferant and analyte concentrations that have been used in the Albumin scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[24,25]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Albumin interference scanning test is appropriate, is determined as ±10%.[26]

In Albumin test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	Albumin Target (g/dL)	N*	Observed Recovery %
Hemoglobin 990 mg/dL	4.29	3	106

Bilirubin 19.5 mg/dL	4.14	3	107
Lipemi 2773 mg/dL	4.17	3	109

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[25]

Annotation:

- It has been reported in the literature that bilirubin, mild to moderate lipemia and salicylate do not affect BCG methods.[4]
- Heparin causes a positive interaction with the BCG method. This interaction can be eliminated by adding a certain amount of hexadimethrin bromide to the BCP reagent.[27]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[25]
These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD**:** For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.
H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.
P264 :Wash your hands properly after using.
P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 :Wash with plenty of water and soap if it contacts with skin.
P333+P313 :Seek medical help if it irritates your skin or develops rash.
P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal

P501 :Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests