Proti 2

Colorimetric method for the determination of Total Proteins and Albumin in serum

SUMMARY

Proteins are macromolecular organic compounds widely distributed in the body and are essential for life. They act as structural and transport elements and also appear as enzymes, hormones, antibodies, coagulation factors, etc.
Albumin is the most abundant protein in serum. One of its most important functions is to enable the transportation of fatty acids, steroid hormones, bilirubin, and catecholamines, which in their free form are not soluble in a water medium.
Albumin concentration in plasma significantly influences on the stability of the coloidosmotic pressure, which is related to its relatively low molecular weight and its high net charge.
Under pathological conditions such as renal loss, malnutrition, long-term infections, etc., hypoproteinemias may appear; while hyperproteinemias are observed with multiple myeloma, bacterial endocarditis and hemoconcentrations of diverse origins.
Both situations are usually accompanied by hypoalbuminemias. Abnormal albumin increases are occasional and are mostly related with dehydration that produces the concomitant increase in the plasma proteic content.

PRINCIPLE

Total Proteins Determination:
Protein peptidic bonds react with the cupric ion in alkaline medium, rendering a violet complex with a maximum absorption at 540 nm, being its intensity proportional to the total proteins concentration of the sample.

Albumin Determination:
Albumin specifically reacts -without previous separation- with the anionic form of the 3,3',5,5'-tetrabromo cresolsulfon phtalein (BCP), in the presence of an excess of dye in buffered medium at pH 3.8. The absorbance increase at 625 nm, related to the reagent Blank, is proportional to the albumin quantity present in the sample.

PROVIDED REAGENTS

A. Reagent A: 13 mmol/l EDTA/Cu complex in 875 mmol/l NaOH and alkyl aryl polyether (AAP).
B. Reagent B: 3,3',5,5'-tetrabromo cresolsulfon phtalein (BCP) solution (in polyoxyethylene lauryl ether).
S. Standard: bovine albumin and globulins solution with known proteins and albumin titers. Check the given values on the label, since they are lot specific.

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.
Reagent A: corrosive. H315+H320: Causes skin and eye irritation. P262 Do not get in eyes, on skin, or on clothing. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352 IF ON SKIN: Wash with plenty of soap and water. P280 Wear protective gloves/ protective clothing/eye protection/face protection.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: are stable at room temperature (< 25ºC) until the expiration date shown on the box.
Standard: stable at room temperature (no higher than 25ºC) until the expiration date shown on the box. Refrigerate after opening.

INSTABILITY OR DETERIORATION OF REAGENTS

Variations in the pH of the reagents may cause their deterioration. Do not exchange the bottle caps.
Any variation in the organoleptic characteristics of the Standard may indicate its deterioration.

SAMPLE

Serum
a) Collection: obtain non-hemolyzed serum.
b) Additives: not required.
c) Known interference substances:
- In Total Proteins determination no interferences from bilirubin up to 100 mg/l, nor from mild hemolysis are observed. Turbidity caused by chylomicrons has never been observed.
- In the Albumin determination no interferences from moderate hemolysis, bilirubin up to 200 mg/l nor from lipemia up to 20 g/l are observed. No previous deproteinization is required, since globulins do not interfere in this test.

See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: if the serum is not immediately tested, it can be stored up to 3 days in refrigerator (2-10ºC) or a week in freezer.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer or photocolorimeter.
- Micropipettes and pipettes to measure the stated volumes.

- Tubes or spectrophotometric cuvettes.
- Water bath at 37°C (for Total Proteins).
- Stopwatch.

• TOTAL PROTEINS DETERMINATION

ASSAY CONDITIONS

- Wavelength: 540 nm in spectrophotometer or photocolorimeter with green filter (520-560 nm).
- Reaction Temperature: 37°C
- Reaction Time: 15 minutes
- Sample Volume: 50 ul
- Reagent A Volume: 3.5 ml
- Final Reaction Volume: 3.55 ml

PROCEDURE

In three test tubes labeled B (Blank), S (Standard) and U (Unknown), place:

	B	S	U
Distilled Water	50 ul	-	-
Standard	-	50 ul	-
Sample	-	-	50 ul
Reagent A	3.5 ml	3.5 ml	3.5 ml

Mix with rod. Incubate for 15 minutes at 37°C. Read in spectrophotometer at 540 nm or in photocolorimeter with green filter (520-560 nm) setting the instruments to zero O.D. with the Reagent Blank.

STABILITY OF FINAL REACTION

The reaction color is stable for 12 hours, thus readings should be performed within this period.

• ALBUMIN DETERMINATION

ASSAY CONDITIONS

- Wavelength: 625 nm in spectrophotometer or photocolorimeter with red filter (620-650 nm).
- Reaction Temperature: 15-28°C
- Reaction Time: 10 minutes
- Sample Volume: 10 ul
- Reagent B Volume: 3.5 ml
- Final Reaction Volume: 3.51 ml

PROCEDURE

In three photocolorimeter test tubes labeled B (Blank), S (Standard) and U (Unknown), place:

	B	S	U
Standard	-	10 ul	-
Sample	-	-	10 ul
Reagent B	3.5 ml	3.5 ml	3.5 ml

Mix with rod. Keep the tubes between 15 and 28°C for 10 minutes. Read in spectrophotometer at 625 nm or in photocolorimeter with red filter (620-650 nm) setting the instruments to zero O.D. with the Reagent Blank.

STABILITY OF FINAL REACTION

The reaction color is stable for 20 minutes, thus readings should be performed within this period.

CALCULATIONS

Using the Standard as indicated in PROCEDURE, the calculations are performed as follows:

$$\text{Total Proteins (g/dl)} = U \times f \qquad f = \frac{\text{T.P. (g/dl)}}{S}$$

$$\text{Albumin (g/dl)} = U \times f \qquad f = \frac{\text{Alb. (g/dl)}}{S}$$

$$\text{A/G Ratio} = \frac{\text{Albumin (g/dl)}}{\text{T.P. (g/dl)} - \text{Alb. (g/dl)}}$$

CALIBRATION CURVE

To check that the colorimeter has a linear response in the wavelengths indicated for the reactions, a calibration curve can be prepared with increasing quantities of Standard (e.g. 50 and 100 ul for Total Proteins; 10 and 20 ul for Albumin) and a reagent volume of 3.5 ml in every case. If the value obtained for the second tube differs more than 5% from the ones calculated in reference to the first tube reading, the calibration curve must be used for the calculations.

QUALITY CONTROL METHOD

Each time the test is run, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known total proteins and albumin concentration.

REFERENCE VALUES

The contents of total proteins and albumin was determined in serum of healthy individuals of both sexes, with normal diet, ages between 17 and 40. The following ranges were obtained:

Total Proteins: 6.1 to 7.9 g/dl
Albumin: 3.5 to 4.8 g/dl
A/G Ratio: 1.2 to 2.2

It is recommended that each laboratory establishes its own reference values.

SI SYSTEM UNITS CONVERSION

Total proteins (g/dl) x 10 = Total proteins (g/l)
Albumin (g/dl) x 10 = Albumin (g/l)

PROCEDURE LIMITATIONS

See Known interference substances under SAMPLE.
- Total Proteins Determination:
  Plasma can be used as sample, but the result of the pro-

teinemia will be increased in 0.2 g/dl due to the presence of fibrinogen, which is not considered in the definition of the Total Proteins.

- Albumin Determination:

  If the reaction is performed between 15 and 28°C a factor can be used for the calculations. Out of this range, the kinetic of the reaction changes and the color development is not completed.

  The system should be standardized with the provided Standard, as lyophilized and serum pools respond in a different manner.

  If an autoanalyzer is used in order to obtain a better performance, it is recommended to Wiener lab's **Albúmina AA**.

PERFORMANCE

a) Reproducibility: when replicates of the same samples were assayed on different days the following results were obtained:

Total Proteins

Level	S.D.	C.V.
4.6 g/dl	± 0.023 g/dl	0.49 %
5.8 g/dl	± 0.023 g/dl	0.40 %
7.0 g/dl	± 0.029 g/dl	0.41 %

Albumin

Level	S.D.	C.V.
2.6 g/dl	± 0.079 g/dl	3.0 %
3.7 g/dl	± 0.071 g/dl	1.9 %
5.5 g/dl	± 0.070 g/dl	1.3 %

b) Recovery: by adding known quantities of albumin and globulins to different samples a recovery of 96 to 103% was obtained for Total Proteins and between 98 and 100% for Albumin.

c) Detection Limit: it depends on the photometer and the wavelength. According to the sensitivity required for a ΔA minimum of 0.001, the smallest detectable concentration change will be of 0.02 g/dl for Total Proteins and 0.01 g/dl for Albumin.

d) Linearity: the reaction is linear up to 12 g/dl for Total Proteins and up to 6 g/dl for Albumin.

WIENER LAB PROVIDES

Kit for 140 Total Proteins and Albumin tests with Standard (Cat. 1690001).

Wiener lab. separately provides:

Proti-2 Suero Patrón: 1 x 1.8 ml (Cat. 1690004).

REFERENCES

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Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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