



Proteínas Totales

AA

Colorimetric method for the determination of total proteins 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. In plasma, proteins help to maintain the circulating fluid volume, transporting relatively insoluble substances, and they act in the inactivation of toxic compounds and in the defense against invasive agents.

The determination of total proteins is useful to monitor changes caused by different diseases. 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 hemoconcentration of diverse origins.

PRINCIPLE

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.

PROVIDED REAGENTS

A. Reagent A: 13 mmol/l EDTA/Cu complex in 875 mmol/l sodium hydroxide and alkyl aryl polyether (AAP).

NON-PROVIDED REAGENTS

Wiener lab.'s **Calibrador A plus / Proti 2 Suero Patrón**.

INSTRUCTIONS FOR USE

Reagent A: ready to use.

WARNINGS

The Reagent is for "in vitro" diagnostic use.

The Reagent A is 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. 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.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagent: is stable at room temperature until the expiration date shown on the box.

SAMPLE

Serum

a) Collection: obtain non-hemolyzed serum.

b) Additives: not required.

c) Known interference substances: no interference from bilirubin up to 100 mg/l, nor from mild hemolysis is observed. Turbidity caused by chylomicrons has never been observed. 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 (2-10°C) or a week in freezer.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer or photocolormeter.
- Micropipettes and pipettes to measure the stated volumes.
- Tubes or spectrophotometric cuvettes.
- Water bath at 37°C.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 540 nm in spectrophotometer or photocolormeter with green filter (520-560 nm).
- Reaction Temperature: 37°C
- Reaction Time: 15 minutes
- Sample Volume: 20 ul
- Reagent A Volume: 2.0 ml (See PERFORMANCE)
- Final Reaction Volume: 2.02 ml

PROCEDURE

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

	B	S	U
Calibrador / Suero Patrón	-	20 ul	-
Sample	-	-	20 ul
Reagent A	2.0 ml	2.0 ml	2.0 ml

Mix with rod. Incubate 15 minutes at 37°C. Read in spectrophotometer at 540 nm or in photocolormeter 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.

CALCULATIONS

Using the **Suero Patrón** as indicated in PROCEDURE, the calculations are performed as follows:

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

*Total proteins concentration in **Calibrador A plus** or **Suero Patrón**

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

Calibration curve

To check that the colorimeter has a linear response in the wavelength indicated for the reaction, a calibration curve can be prepared with increasing quantities of **Suero Patrón** (e.g. 20 and 40 μ l) and a reagent volume of 2.0 ml in every case. If the value obtained for the second tube differs more than 5% from the one calculated in reference to the first tube reading, the calibration curve must be used for the calculations.

SI SYSTEM UNITS CONVERSION

Total proteins (g/dl) $\times 10$ = Total proteins (g/l)

QUALITY CONTROL METHOD

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

REFERENCE VALUES

The content of total proteins 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

A/G Ratio: 1.2 to 2.2

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

PROCEDURE LIMITATIONS

See Known interference substances under SAMPLE.

Plasma can be used as sample, but the result of the proteinemia will be increased in 0.2 g/dl due to the presence of fibrinogen, which is not considered within the Total Proteins definition.

PERFORMANCE

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

Level	S.D.	C.V.
4.6 g/dl	± 0.022 g/dl	0.49 %
5.8 g/dl	± 0.023 g/dl	0.56 %
7.0 g/dl	± 0.028 g/dl	0.39 %

b) Recovery: by adding known quantities of albumin and globulins to different samples a recovery of 96 to 103 % was obtained.

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 0.01 g/dl.

d) Linearity: the reaction is linear up to 17 g/dl. If the instrument used for the reading has a low photocolometric sensitivity, 20 μ l of sample with 1.5 ml of Reagent A can be used. In this case the linearity reaches 12 g/dl of total proteins.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use.

For calibration, it can be used Wiener lab.'s **Calibrador A plus**.

WIENER LAB PROVIDES

- 10 x 20 ml (Cat. 1009327).
- 10 x 20 ml (Cat. 1009630).
- 10 x 20 ml (Cat. 1009273).
- 6 x 120 ml (Cat. 1690009).

REFERENCES

- Gasbarro, L.; Bandinelli R. & Tomassini, G. - Clin. Chim. Acta 36/1:275 (1972).
- Strickland, R.D.; Freeman, M.L. & Gurule E.T. - Anal. Chem. 33:545 (1961).
- Pastewka, J. W. & Ness, A.T. - Clin. Chim. Acta 12:523 (1965).
- Peters, T. Jr. - Clin. Chem. 14:1147 (1968).
- Henry, R., Sobel, C. & Berkman, S. - Anal. Chem. 29/10:1491 (1957).
- Kachmar, J.F. - Fundamentals of Clinical Chemistry - Tietz, Saunders, pág. 177 (1970).
- Rojkin, M.L.; Olguin de Mariani, M.C.; Drappo, G.A. y Sosa, C.F. - Bioq. del Atlántico VI/63: 1931 (1974).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 3rd ed., 1990.

Symbols

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



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



Authorized representative in the European Community



"In vitro" diagnostic medical device



Contains sufficient for <n> tests



Use by



Temperature limitation (store at)



Do not freeze



Biological risks



Volume after reconstitution



Contents



Batch code



Manufactured by:



Harmful



Corrosive / Caustic



Irritant



Consult instructions for use



Calibrator



Control




Positive Control



Negative Control



Catalog number

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