

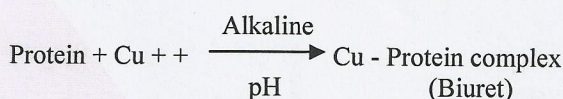


TOTAL PROTEIN

INTRODUCTION AND PRINCIPLE:

Through osmotic pressure, Serum Protein is involved in the maintenance of normal distribution of water between blood and tissues. The several fractions of Serum Protein vary independently and widely in disease. Low Protein is primarily caused by malnutrition, impaired synthesis, loss (as by hemorrhage), or excessive Protein catabolism, Elevated Protein levels are caused mainly by dehydration.

The enzymatic reaction sequence employed in the assay of Total Protein is as follows:



REAGENTS:

Total Protein Reagent contains the following:

1. Total Protein Reagent : Ready – To – Use
2. Total Protein standard : Ready – To – Use

STORAGE & STABILITY

Total Protein reagent is stored at Room temperature ($15^{\circ} - 25^{\circ}\text{C}$) and the standard at $2^{\circ} - 8^{\circ}\text{C}$.

Avoid Contamination of Ready – To – Use Reagent. Always use fresh pipette tips. Keep always the caps tightly closed.

SPECIMEN COLLECTION:

1. test specimens should be serum and free from hemolysis.
2. Cross hemolysis will cause elevated results because of the released hemoglobin, as well as the increase in background color
3. Lipemic sera cause elevated results and should be run with a serum blank. Place 1.0ml 0.9% saline in test tube. Add 0.02 ml (20 ul) sample. Zero spectrophotometer with 0.9% saline. Read and record absorbance of serum blank. Subtract blank absorbance from test absorbance. Calculate as usual.

4. Samples with bromsulfophthalein (BSP) will result in falsely elevated results.
5. Protein in serum is stable for one week at Room Temperature and for at least one month refrigerated, when guarded against evaporation.

LIMITATIONS:

1. The Reagent is linear up to 15.0 g/dl Samples with values above 15.0 g/dl should be diluted 1:1 with 0.9% saline, re-run and result multiplied by two.
2. The Biuret procedure is not sensitive at low ranges (<1 g/dl). Do not use for urine or spinal fluid.

PROCEDURE:

METHOD	: End Point
WAVE LENGTH	: 546 nm
TEMPERATURE	: 37°C
INCUBATION	: 5 mins.
STANDARD	: 4 g/dl. (refer the vial label)

Pipette into cuvettes	Macro	Semi-Macro
Reagent	1000 ul	500 ul
Sample/Standard	20 ul	10 ul

Mix & incubate for 5 minutes at RT and read the absorbance of all cuvettes at 520 – 560 nm within 60 minutes, against reagent blank.

CALCULATIONS:

Abs. of sample

$$\frac{\text{Abs. of sample}}{\text{Abs. of Standard}} \times \text{Concentration of Standard} = \text{Total Protein (g/dl)}$$

NOTE: Gross hemolysis & lipemic sera give elevated values and should be run with reagent blank.

EXPECTED VALUES : 6.2 – 8.5 g/dl

LINEARITY : 12.0 g/dl

REFERENCES:

1. Peters, T. and Biamonte, G.T., Selected Methods for the Small Clinical Chemistry Laboratory; Faulber, W.R. and Meites, S.,
2. Doumas, B.T., et al.: Clin. Chem. 27: 1642 (1981).
3. Gornall, A.J. Biol. Chem., 177, (1981)