

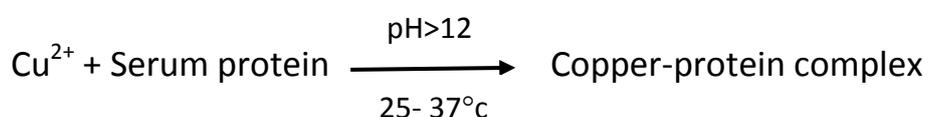
Total Protein

1. Clinical importance.

The serum content of the soluble proteins, those circulating in extracellular and intracellular fluids, has been used as a marker to aid in clinical diagnosis. The main diagnostic tests are those measuring serum total protein and serum albumin. Collectively, serum total protein including albumin is mainly involved in the maintenance of normal water distribution between tissues and the blood and responsible for maintaining the oncotic pressure of plasma and is used to transport many substances including macromolecules. Hyperproteinemia or hyperalbuminemia, usually occurs during multiple myeloma caused by high levels of the monoclonal immunoglobulins, dehydration, excessive water loss, as in severe vomiting, diarrhea, Addison's disease or diabetic acidosis. The hemoconcentration, decrease in the volume of plasma water, is reflected as a relative hyperproteinemia since concentration of all the individual plasma proteins are increased to the same degree. Hypoproteinemia or hypoalbuminemia usually occurs in edema, malnutrition, nephrotic syndrome, malabsorption and severe liver cirrhosis. Since albumin is present in such high concentration low levels of this protein alone may also cause hypoproteinemia.

2. Principle.

In the biuret reaction, a chelate is formed between the Cu^{2+} ion and the peptide bonds of the proteins in alkaline solutions to form a violet colored complex whose absorbance is measured photometrically. The intensity of the color produced is proportional to the concentration of protein in the sample.



3. Samples.

Serum or heparinized plasma. Total protein is stable in serum and plasma for 1 week at room temperature, for at least 1 month refrigerated at 2-8°C., and for up to 2 months at -20°C.

4. Procedure.

1. Bring reagents and samples to room temperature.
2. In disposable test tube add 1 ml of monoreagent (reagent 1) and label the tube as sample.
3. Add 20 µl of serum to the sample tube.
4. In other disposable test tube add 1 ml of monoreagent (reagent 1) and label the tube as standard.
5. Add 20 µl of standard solution (reagent 2).
6. Mix and let the tubes 5 minutes at 37°C.
7. Read the absorbance (A) of the samples and the standard at 540 nm against the reagent blank. The color is stable for at least 1 hours.
8. Calculate the concentration of protein from the equation:

$$\text{protein concentration g/dl} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}}$$

A : Absorbance.

C: concentration.

Note: Samples with concentrations higher than 12 g/dl should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

Normal values for protein concentration are:

Adults: 6.6 - 8.7 g/dl.

Prematures: 3.6 - 6.0 g/dl.

Newborns: 5.3 - 8.9 g/dl.