

Estimation of total protein in blood serum by biuret reaction

Principle:

Proteins and peptides, similarly to biuret, react with cupric ions in alkaline solutions to form a violet complex suitable for the photometric determination.

Procedure:

1. Pipette into three test tubes:

	sample	standard	blank
sample (ml)	0.1	-	-
standard (ml)	-	0.1	-
physiol. sol. (ml)	-	-	0.1
biuret reagent (ml)	5,0	5.0	5.0

2. Mix properly and allow to stand for 30 min at room temperature.
3. Measure the absorbances of the sample and the standard at 546 nm against the blank.
4. Calculate the total protein concentration:

$$\text{Total protein concentration (g/l)} = \frac{\mathbf{A \text{ sample}}}{\mathbf{A \text{ standard}}} \times \mathbf{c \text{ standard}} \text{ (70 g/l)}$$

Estimation of albumin in blood serum

Principle:

Sulphonphthalein dyes as bromocresol purple or bromocresol green yield with albumin in the presence of detergents in a blue-green complex suitable for the photometric determination.

Procedure:

1. Pipette into three test tubes:

	sample	standard	blank
sample (ml)	0.02	-	-
standard (ml)	-	0.02	-
distilled water (ml)	-	-	0.02
reagent (ml)	2.0	2.0	2.0

2. Mix properly and allow to stand for 10 min at room temperature.
3. Measure the absorbances of the sample and the standard at 600 nm against the blank.
4. Calculate the albumin concentration:

$$\text{Albumin concentration (g/l)} = \frac{A \text{ sample}}{A \text{ standard}} \times c \text{ standard (40 g/l)}$$

5. Calculate the A/G ratio (albumin/globulin ratio):

$$A/G = \frac{c \text{ albumin}}{c \text{ total protein} - c \text{ albumin}}$$

Estimation of C-reactive protein (CRP)

CRP is the classic acute phase protein, one of the first to be recognized. An increase in serum or plasma almost invariably indicates the presence of *inflammation*, most markedly *bacterial infections*. In addition, increased CRP concentrations accompany *tissue necrosis* and *malignancies*, reflecting severity of the disease and the mass of affected tissue. In an acute event, plasma CRP is elevated after 6 h, reach a peak at 48 h and decline with a half time of about 48 h.

CRP is composed of five polypeptide subunits each of 206 amino acid residues, which places CRP in the family of pentraxins, proteins with immune defence properties found in all vertebrates and most invertebrates. CRP is synthesised rapidly in the liver following induction and at the peak of an acute phase response its synthesis may account for as much as 20% of the liver protein synthetic capacity.

The biological functions of CRP are its ability to bind a wide range of endogenous and exogenous substances and then to facilitate their removal from blood and tissues by opsonization (ie. by enhancing the process of phagocytosis or killing by specific lymphocytes). CRP binding to host cells only occurs when the normal structure of the lipid bilayer has been disrupted. On the contrary, the binding to the cell wall in bacteria and other parasites will occur to live, intact organisms. CRP binding may even crosslink some ligands to precipitate them and localize in the tissues.

Methodology:

CRP is determined mostly by an immunoturbidimetric method. The specimen (serum plasma) is incubated in the presence of specific antibodies against human CRP (antiserum, monoclonal antibodies) and the extent of immunoprecipitation is quantified as turbidity at 700 nm.

Reagents: Goat antiserum against human CRP (10%) in Tris buffer (0.1 mol/L, pH 7.6)

Procedure:

- (1) To 0.05 ml of serum add 2.0 ml of antibody solution, mix and allow to stand **at 37°** for 10 min.
- (2) Read absorbance of a turbid solution against water at 700 nm.
- (3) Plot the absorbance value onto the calibration graph and read the concentration of CRP in mg/L.

