Practice No. 2: DETERMINATION OF THE TOTAL PROTEIN CONCENTRATION IN THE SERUM SAMPLE USING THE AUTOMATED PHOTOMETRIC ANALYZER BS 200

THEORETICAL PREPARATION FOR PRACTICE

Total protein

Determination of total serum protein is a common and affordable method. The total serum protein assay provides approximate information on biosynthesis, protein utilization, and their excretion. Changes in the composition of serum proteins give rise to various diseases, but only some are manifested by a deviation in total protein values. Total protein is involved in maintaining oncotic pressure and thus maintaining the volume of body fluids (especially albumin). It has defense functions against pathogens (immunoglobulins, complement, acute phase proteins (eg CRP)), antioxidant functions (defense against reactive oxygen species) - eg albumin, ceruloplasmin, transport functions - for hormones (cortisol, thyroid hormones) for elements (Ca, Mg, Cu, Fe), bilirubin. The total protein participates in blood clotting (coagulation factors) and is part of enzymes and inhibitors.

The concentration of total serum protein may be affected by the following basic factors:

- Hydratation of the organism;
- Changing the biosynthesis of one or more specific proteins;
- The rate of loss of one or more specific proteins.

Hypoproteinemia

Hypoproteinemia stands for *reduced protein concentration in the serum*.

Absolute hypoproteinemia occurs due to *decreased serum protein* in:

- Increased losses;
  - through kidneys;
  - through gastrointestinal tract (eg. intestinal inflammation);
  - through skin (burns);
  - by bleeding;
  - to „third space“ (eg., the abdominal cavity at ascites);
- reduced liver proteosynthesis (chronic liver disease);
- inadequate intake of protein by food during nutrition disorders.

Relative hypoproteinemia retains normal amounts of protein, which are "diluted" due to retention of water and electrolytes (hyperhydration status). Concentrations of individual proteins are reduced in the same ratio.

Hyperproteinemia

Hyperproteinemia means an *increased concentration of total serum protein*. It occurs in infections, inflammation, dehydration, hemolysis, trauma, thyroid hyperfunction, iron deficiency, the most frequent cause is the decrease of body water volume, in case of water loss.

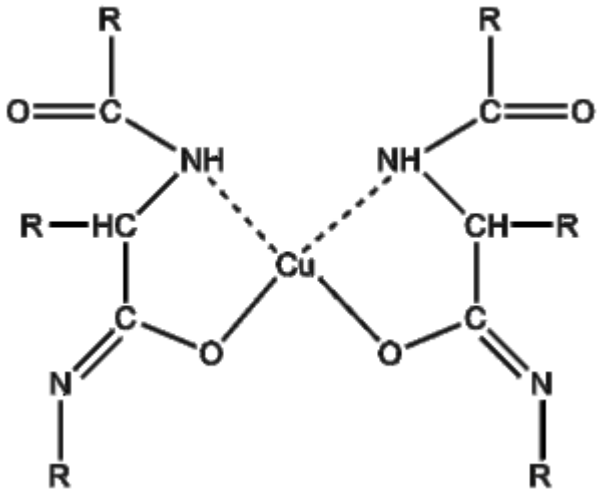
Absolute hyperproteinemia is *an increase in protein concentration* caused by the increased synthesis of some specific proteins, eg immunoglobulins (plasmocytoma).

Relative hyperproteinemia arises as a result of *dehydration of the organism* with insufficient intake or excessive fluid loss (severe diarrhea, vomiting). The total amount of protein is retained and the concentration of individual proteins is increased proportionally.

Reference range: Total serum protein concentration (S-total protein): 65 -85 g / L

METHOD PRINCIPLE

The principle of total protein determination
Total serum protein is determined by **the biuret reaction**.



Protein complex with copper in alkaline environment
In an alkaline environment in the presence of copper salts, the proteins give a purple color, suitable for photometric determination. In a simplified way it can be said that complex compounds of Cu^{2+} with peptide bonds are formed. The resulting complex strongly absorbs light in the wavelength range of 540-560 nm. The color intensity of the complex is measured photometrically and is directly proportional to the protein concentration. The biuret reaction generally provides substances containing at least two peptide bonds (-CO-NH-) or two -CO-NH_2 in the molecule. Therefore, the reaction is not specific to protein only. The simplest compound reacting with copper salts in an alkaline environment is biuret containing two peptide bonds. Amino acids and dipeptides do not react with copper salts.
The biuret component is ***copper sulfate***, which provides Cu^{2+} to form complexes with peptide bonds, and an alkalizing component (hydroxide) that converts the peptide bond to the enolform to allow for the participation of the oxygen atoms in the complex. The other ingredients of the reagent are sodium potassium tartrate, which prevents Cu^{2+} from precipitation of Cu^{2+} to Cu $(OH)_2$, and potassium iodide, which protects Cu^{2+} from autoreduction. The sensitivity of the biuret method is around 1 - 10 g protein / L.

DESCRIPTION OF THE BS-200 PHOTOMETER

The automated BS-200 (Mindray, China) photometer, which consists of a cuvette, a reagent carousel for reagents and sample preparation (tempered at 4 ± 1 ° C) and an optical detector, will be used to measure total serum protein. The light source is a halogen-tungsten lamp. Sample transfer and reagents are provided by a robotic arm with a dispensing needle. The contents of the cuvettes are mixed with an automatic stirrer immediately after addition of a reagent (eg. 200 µl) or sample (eg.4 µl). Contamination is minimized by flushing both the dispensing needle and the stirrer with distilled water. For detection, following wavelengths can be used: 340, 405, 450, 510, 546, 578, 630, 670 nm. The device is fully controlled by BS 200 software (Mindray, China).

MEASUREMENT PROCEDURE FOR AUTOMATED PHOTOMETER BS 200

The 200 µl biuret reagent (100 mM sodium potassium tartrate, 100 mM NaOH, 15 mM KI, 6 mM CuSO4) is pipetted into the cuvette and then is mixed with 4 µl of sample. After 5 min of incubation at 37 ° C, absorbance at λ = 546 nm is measured. The absorbance of the reagent itself and the absorbance value after a 5 minute incubation with the sample are used for the calculation. The reagent vial has a biuret reagent (blue color) already in the instrument. That is why we are just preparing samples.

MATERIAL

Unknown sample: Lyonorm calibrator which contains the total protein of unknown concentration 4 × diluted. The calibrator is delivered freeze-dried to the manufacturer and ready for use just before training by reconstituting with deionized water as instructed by the manufacturer. The expiration time of the reconstituted calibrator will be indicated on each vial

The 20 g / l bovine serum albumin (BSA) standard and **the unknown sample** (4 × diluted) are prepared in the micro-tube.

Distilled water for standard dilution, micro-tubes, adjustable pipette 100 -1000 µl.

WORKING PROCEDURE:

1. Place 5 micro-tubes (1.5 ml) and label them with the letter (A, B, C, and serial number (eg A1, A2, A3, A4, A5, B1, B2, B3 etc), unknown sample and BSA standard (20g / l)
2. Prepare the dilution line from the concentrated BSA standard (20 g / l) by half dilution
3. Pipette 200 µl of water first into the micro-tubes No. 2, 3 and 4.
4. Pipette 200 µl of BSA standard (20g / l) into the micro-tube No. 1 and pipette the same volume of the standard into micro-tube No. 2, where 200 µl of water has already been added and the micro-tube content is then mixed by pipetting to give a BSA solution of half concentration (10 g / l). Then from the micro-tube No. 2, pipette 200 µl of BSA solution (10 g/ l) into micro-tube No. 3 (in which we already have 200 µl of water) and again mix the micro-tube content by pipetting. This gives a BSA solution of half concentration (5g / l). We have 200 µl of water already in the micro-tube 4 and it is the blank. Add 200 µl of unknown sample to micro-tube No. 5.

Movement to the central laboratory

5. Place the micro tubes in the BS 200 photometer and start the measurement.
6. Measure the absorbance at a wavelength of 546 nm (measurement takes 18 minutes).
7. After finishing the measurement, absorbance values can be written into the table

Movement back to the original lab

From the measured absorbance values and the known albumin concentrations, we make a calibration curve in the Excel program and calculate the concentration of the 4x diluted unknown sample from the equation of the calibration line.

Microtube	**Total Protein concentration (x-axis)**	A_{546} (derived absorbance in 10 000 units	**A_{546} SAMPLE - A_{546}BLANK (y-axis)**	**Measured concentration**
1	Standard 1 concentration **20 g/l**			
2	Standard 2 concentration **10 g/l**			
3	Standard 3 concentration **5 g/l**			
4	Blank (water) **0 g/l**			
5	Unknown sample (calibrator, 4 × diluted)			4 x diluted unknown sample concentration is subtracted from the calibration curve

PROTOCOL (SCHEME):

The importance of total serum protein determination.
When does hyperproteinemia and hypoproteinemia occur?
Reference range of total protein concentration in human serum.
The principle of total protein determination by biuret reaction.
Measuring procedure on an automated photometer BS 200.
A filled-in table and graph of the calibration curve.
Calculation of unknown sample concentration.