Instructions for the practical lesson on biochemistry

Topic: Proteins in serum and urine

Task 1: Estimation of total serum protein with the biuret method

Reagents:

A commercial kit Celková bílkovina liquid 500 S made by Erba -Lachema Diagnostika s.r.o., is used for analysis.

1. Biuret reagent: Copper sulfate 12.0 mmol/l
   Sodium-potassium tartrate 31.9 mmol/l

   Sodium hydroxide 0.6 mol/l
   Potassium iodide 30.1 mmol/l

2. Standard protein solutions:
   Standard 1 20 g/l
   Standard 2 40 g/l
   Standard 3 60 g/l
   Standard 4 80 g/l
   Standard 5 100 g/l

3. Serum sample of unknown concentration

4. Deionized water

Procedure:
Prepare and mark 7 short test tubes. Measure the solutions according to the table:

Measure in ml	**Tube 1**	**Tube 2**	**Tube 3**	**Tube 4**	**Tube 5**	**Tube 6**	**Tube 7**
	Standard 1 20 g/l	Standard 2 40 g/l	Standard 3 60 g/l	Standard 4 80 g/l	Standard 5 100 g/l	Unknown sample	Blank
Standard 1–5	0.02	0.02	0.02	0.02	0.02	–	–
Unknown sample	–	–	–	–	–	0.02	–
Deionized water	–	–	–	–	–	–	0.02
Biuret reagent	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Mix the tubes and incubate 10 minutes at the ambient temperature. Protect from direct light. After the incubation measure the absorbances of standard solutions 1 to 5 and the unknown sample against the blank (Tube No. 7) in 1 cm cuvette at the wavelength 540 nm.

Construct a calibration graph using concentrations of standard solutions 1–5 and the corresponding absorbances. Plot a straight line going through the origin. Read the protein concentration in the unknown sample from the calibration graph.

In the conclusion, compare the total protein concentration measured in the unknown sample with the reference range.

Task 2: Estimation of serum concentration of albumin

Reagents:

A commercial kit Albumin Biosystems S.A. (from Spain) is used for analysis.

1. Reagent

Bromcresol green	0.27 mmol/l
Acetate buffer pH 4.2	100.0 mmol/l
Detergent	2.0 g/l
Sodium azide 	0.5 g/l

1. Standard solution of albumin 50 g/l
2. Serum sample of unknown concentration
3. Deionized water

Procedure:

Prepare and mark 3 short test tubes. Measure the solutions according to the table:

Measure in ml	**Test tube 1 Sample**	**Test tube 2 Standard**	**Test tube 3 Blank**
Reagent	1.0	1.0	1.0
Serum	0.01	–	–
Standard	–	0.01	–
Deionized water	–	–	0.01

Mix the tubes and incubate 1 minute at the ambient temperature. Measure the absorbances of serum and standard against the blank in 1 cm cuvette at 630 nm.

Calculate the albumin concentration in the unknown sample:

$$\text{S-albumin (g/l)} = \frac{A_{sample}}{A_{standard}} \times \text{standard concentration}$$

In the conclusion, compare the albumin concentration measured in the unknown serum sample with the reference range.

Task 3: Evaluation of electrophoresis of serum proteins

Authentic electrophoreograms of serum proteins are available.

Evaluate three of them. Redraw their densitometric records and try to determine what type of dysproteinemia is present.

Task 4: Qualitative estimation of protein in urine

Reagents:

1. Sulfosalicylic acid dihydrate  200 g/l
2. Sample of urine with protein
3. Sample of urine without protein
4. Diagnostic strip with reaction zone for protein

Procedure:

Perform the qualitative test for protein with both methods in both urine samples; record and compare your observations.

Test with sulfosalicylic acid: to about 1 ml of sample add a few drops of aqueous sulfosalicylic acid. Appearance of turbidity or even precipitate indicates presence of protein. The change is best evaluated against a black background or a page with printed text. Perform the reaction with both the urine samples with protein and without protein.

Diagnostic strip test: Completely immerse the reagent pad into urine specimen for 1 second. Place the strip on an absorbent support to remove the excess of urine. Read the result after about 60 seconds by comparing the color of the test pad to the scale on the tube label. Again perform with both the samples with/without protein.

Task 5: Quantitative estimation of protein in urine

Reagents:

A commercial kit Celková bílkovina 600 M made by SKALAB Svitavy is used for analysis.

1. Buffer:	Sodium benzoate 	6.94 mmol/l
	Succinic acid	100.0 mmol/l
	Sodium molybdate	0.12 mmol/l
	Sodium oxalate	2.09 mmol/l
	Detergents and stabilizers	
2. Chromogen:	Pyrogallol red	0.14 mmol/l
	Stabilizers	

3. Standard protein solution 2.0 g/l

4. Unknown sample of urine

Procedure:

Prepare and mark 3 test tubes. Measure the solutions according to the table:

Measure in ml	**Test tube 1 Sample**	**Test tube 2 Standard**	**Test tube 3 Blank**
Urine	0.03	–	–
Standard	–	0.03	–
Deionized water	–	–	0.03
Buffer	0.5	0.5	0.5
Chromogen	0.5	0.5	0.5

Mix the tubes and incubate 5 minutes at the ambient temperature. Measure the absorbances of urine and standard against the blank in 1 cm cuvette at 600 nm.

Calculate concentration of protein in urine(U-protein) as well as the loss of protein to urine per 24 hours (dU-protein):

$$\text{U-protein (g/l)} = \frac{A_{sample}}{A_{standard}} \times \text{standard concentration}$$

$$\text{dU-protein (g/24 h)} = \text{U-protein (g/l)} \times \text{diuresis (l/24 h)}$$

In the conclusion, try to decide whether the patient has a pathological proteinuria and evaluate its severity.

Task 6: Evaluation of electrophoresis of urinary proteins

Authentic electrophoreograms of urinary proteins are available.

Evaluate three of them. Draw the positions of the observed protein fractions and try to determine what type of proteinuria is present.