Date ..... Name ..... Group .....

Instructions for the practical lesson on biochemistry

Topic: Non-protein nitrogen compounds

Task 1: Estimation of creatinine in serum and urine

Reagents:

1. **Trichloroacetic acid** 1.22 mol/l
2. **Picric acid** 0.04 mol/l

3. **Sodium hydroxide** 0.75 mol/l
4. **Standard of creatinine 120 µmol/l**.
5. **Serum** – unknown sample
6. **Urine** – unknown sample, diluted **60x**.

Procedure:
The serum sample is subjected to deproteinization prior to the assay – pipette the serum to short centrifugation tube.

Measure in ml:	Serum sample (Tube No 1)	Urine sample (Tube No 2)	Standard (Tube No 3)	Blank (Tube No 4)
Serum	0.5	-	-	-
Urine (diluted)	-	0.5	-	-
Standard	-	-	0.5	-
Distilled water	1.0	1.0	1.0	1.5
Trichloroacetic acid	0.5	0.5	0.5	0.5
Mix all tubes, allow to stand for 5 minutes, and only the test tube with serum centrifuge for 10 minutes at 3,000 rpm in the large centrifuge. Take <u>new</u> four test tubes, and pipette the supernatant, urine, standard and blank from previous tubes:				
Supernatant from tube 1	1.0	-	-	-
Urine (from tube 2)	-	1.0	-	-
Supernatant from tube 3	-	-	1.0	-
Blank (from tube 4)	-	-	-	1.0
Picric acid	0.5	0.5	0.5	0.5
Sodium hydroxide	0.5	0.5	0.5	0.5
Mix and exactly after 20 minutes measure the absorbances of serum, urine and standard against the blank at 505 nm in 1 cm cuvette.				

Calculations:

Concentration of creatinine in serum (S-Creatinine):

$$\text{S-Creatinine (µmol/l)} = \frac{A_{serum}}{A_{standard}} \times c_{standard}\ (\mu mol/l)$$

Concentration of creatinine in the urine (U-Creatinine):

$$\text{U-Creatinine (}\mathbf{mmol/l}\text{)} = \frac{A_{urine}}{A_{standard}} \times c_{standard}\ (\mathbf{mmol/l}) \times \text{Dilution of urine}$$

Daily output of creatinine into urine (dU-Creatinine):

dU-Creatinine (mmol/24 hrs) = U-Creatinine (mmol/l) × Volume of urine (liters/24 hrs)

Task 2: Calculation of clearance of endogenous creatinine

Clearance of endogenous creatinine (Cl_{Cr}):

$$Cl_{Cr}\ (ml/s) = \frac{U \times V}{P}$$

U: Concentration of creatinine in urine (mmol/l)

P: Concentration of creatinine in serum **(mmol/l)**

V: Volume of urine per 24 hours (ml/s)

Clearance of endogenous creatinine corrected to body surface:

a) Calculation of body surface:

$$A = 0.167 \times \sqrt{m \times l}$$

m: Weight of patient in kg

l: Height of patient in m

b) Correction of clearance to body surface:

$$Cl_{Cr}\ \text{corr. (ml/s)} = Cl_{Cr} \times \frac{1.73}{A\ (m^2)}$$

Calculation of creatinine clearance from serum creatinine using the Cockroft & Gault formula:

Estimation of Cl_{Cr} for men:

$$Cl_{Cr}\ (ml/s) = \frac{(140 - \text{Age [years]}) \times \text{Weight [kg]}}{44.5 \times \text{Serum creatinine } (\mu mol/l)}$$

Estimation of Cl_{Cr} for women:

$$Cl_{Cr}\ (ml/s) = 0.85 \times \frac{(140 - \text{Age [years]}) \times \text{Weight [kg]}}{44.5 \times \text{Serum creatinine } (\mu mol/l)}$$

Calculation of fractional excretion and tubular reabsorption of water:

a) Fractional excretion (FE) of water:

$$FE_{H2O} = \frac{P_{creatinine}}{U_{creatinine}}$$

b) Tubular reabsorption (TR) of water:

$$TR_{H2O} = \frac{Cl_{Cr} - V}{Cl_{Cr}}$$

Evaluation and conclusion:
Summarize the obtained results and decide whether any of these parameters indicate impaired renal functions. Compare also the measured and calculated values of clearance – is there a difference that would suggest an inadequate collection of urine?

Task 3: Estimation of urea in serum and urine

Reagents:
Commercial kit for kinetic estimation of urea Bio-La-Test UREA 1000 manufactured by Erba-Lachema s.r.o. is used.

1. **R1 reagent:**
   Tris buffer, 100 mmol/l
   2-oxoglutarate, 5.49 mmol/l
   Urease ≥ 166,6 µkat/l
   L-glutamate dehydrogenase (GLDH) ≥ 63.3 µkat/l
2. **R2 reagent:** NADH 1.66 mmol/l
3. **Working solution**: 4 volumes R1 mixed with 1 volume R2
4. **Standard solution of urea 6.7 mmol/l**.
5. **Serum** – unknown sample (infectious material)
6. **Diluted urine** – unknown sample (infectious material). Diluted **50x**.

Procedure:

Working solution and spectrophotometric cuvettes need to be pre-warmed to 37 °C before analysis.

N.B.: Use distilled water as a blank. First process the cuvette No. 1 (serum sample): Prepare the mixture as directed by the table below, mix well by repeated pipetting, after 30 seconds measure the absorbance A_1, and after exactly 60 seconds measure the absorbance again, obtaining A_2. Then perform the same with the cuvette No. 2 (urine sample), and finally with the cuvette No. 3 (standard).

Measure in ml:	**Cuvette No. 1** Serum sample	**Cuvette No. 2** Urine sample	**Cuvette No. 3** Standard
Serum	0.01	-	-
Urine	-	0.01	-
Standard	-	-	0.01
Working solution	1.0	1.0	1.0
Mix, after 30 seconds measure absorbance A_1 at 340 nm against distilled water.			
Leave the cuvette in the instrument and after 60 seconds measure the absorbance A_2.			

Calculations:

Concentration of urea in serum (S-Urea):

$$\text{S-Urea (mmol/l)} = \frac{(A_1 - A_2)_{serum}}{(A_1 - A_2)_{standard}} \times c_{standard}$$

Concentration of urea in the urine (U-Urea):

$$\text{U-Urea (mmol/l)} = \frac{(A_1 - A_2)_{urine}}{(A_1 - A_2)_{standard}} \times c_{standard} \times \text{Dilution of urine}$$

Daily output of urea into urine (dU-Urea):

dU-Urea (mmol/24 hrs) = U-Urea (mmol/l) × Volume of urine (liters/24 hrs)

Task 4: Estimation of uric acid in serum and urine

Reagents:
Commercial Kit for estimation of uric acid (uricase/peroxidase), BioSystems, is used.

1. **Working solution:**
   Phosphate buffer 10 mmol/l pH 7.8
   Detergent 1.5 g/l
   Dichlorophenolsulfonate 4 mmol/l
   Uricase > 2 nkat/ml
   Ascorbate oxidase > 8.3 µkat/ml
   Peroxidase > 1.66 µkat/ml
   4-aminoantipyrine 0.5 mmol/l
2. **Standard of uric acid 120 µmol/l**
3. **Serum** – unknown sample
4. **Urine** – unknown sample. Diluted **10x**.

Procedure:

Measure in ml:	Serum sample (Tube No. 1)	Urine sample (Tube No. 2)	Standard (Tube No. 3)	Blank (Tube No. 4)
Working solution	1.0	1.0	1.0	1.0
Serum	0.025	-	-	-
Urine	-	0.025	-	-
Standard	-	-	0.025	-
Distilled water	-	-	-	0.025
Mix and incubate 5 minutes at 37 °C. Then measure absorbances of the serum, urine and standard against the blank (tube No 4) at wavelength 520 nm.				

Calculations:

Uric acid in serum (S-Uric acid):

$$\text{S-Uric acid (µmol/l)} = \frac{A_{serum}}{A_{standard}} \times c_{standard}\ (\mu mol/l)$$

Uric acid in the urine (U-Uric acid):

$$\text{U-Uric acid (}\mathbf{mmol/l}\text{)} = \frac{A_{urine}}{A_{standard}} \times c_{standard}\ (\mathbf{mmol/l}) \times \text{Dilution of urine}$$

Daily output of uric acid into urine (dU-Uric acid):

dU-Uric acid (mmol/24 hrs) = U-Uric acid (mmol/l) × Volume of urine (liters/24 hrs)

Task 5: Calculation of clearance and fractional excretion of uric acid

Clearance of uric acid (Cl_{UA}):

$$Cl_{UA}\ (ml/s) = \frac{U_{UA} \times V}{P_{UA}}$$

U_{UA}: Concentration of uric acid in urine (mmol/l)

P_{UA}: Concentration of uric acid in serum (mmol/l)

V: Volume of urine per 24 hours (ml/s)

Fractional excretion of uric acid:

$$FE_{UA} = \frac{U_{UA} \times P_{Cr}}{U_{Cr} \times P_{UA}}$$

U_{Cr}: Creatinine in urine (mmol/l)

P_{Cr}: Creatinine in serum (mmol/l)

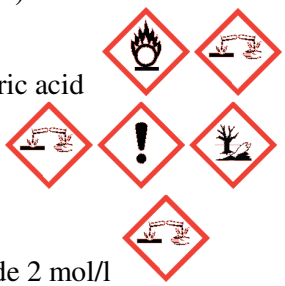
U_{UA}: Concentration of uric acid in urine (mmol/l)

P_{UA}: Concentration of uric acid in serum (mmol/l)

Task 6: Murexide reaction

Reagents:

1. Uric acid (powder)
2. Concentrated nitric acid
3. Ammonia 70 g/l
4. Sodium hydroxide 2 mol/l



Procedure:

Put a small amount of solid uric acid into an evaporating dish and add 2 drops of concentrated nitric acid (Take care when working with concentrated acid!). Place the dish on the top of a beaker with boiling water and wait until the mixture in the dish completely evaporates. Transfer the dish on an asbestos grid and allow it to cool down. Then add 1-2 drops of aqueous ammonia. A purple color of murexide should develop. It turns violet after addition of sodium hydroxide.

Task 7: Solubility of uric acid

Reagents:

1. Uric acid (powder)
2. Hydrochloric acid diluted
3. Sodium hydroxide 2 mol/l

Procedure:

Try to dissolve a small amount of solid uric acid in about 2 ml of distilled water. Add a few drops of NaOH and observe the effect on solubility. Take a portion of the solution to another tube, add a few drops of HCl and again observe the effect on solubility of uric acid.