ÚSTAV LÉKAŘSKÉ BIOCHEMIE A LABORATORNÍ DIAGNOSTIKY 1. LF UK A VFN

Selected examination of kidney and cerebrospinal fluid

Practical lesson in medical biochemistry

General Medicine

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Task 1: Estimation of creatinine in serum and urine

Reagents:

Commercial kit CREATININE, Erba Lachema s.r.o., is used for analysis.

1. Picric acid (5 mmol/l) 
2. Sodium hydroxide (0.183 mol/l) 
3. Standard solution of creatinine (concentration is on the label)
4. Serum – unknown sample
5. Urine – unknown sample (dilution on the label)

Procedure:

Pipette to short glass tubes:

Measure in ml:	Serum (Tube No. 1)	Urine (Tube No. 2)	Standard (Tube No. 3)	Blank (Tube No. 4)
Sodium hydroxide	0.8	0.8	0.8	0.8
Serum	0.05	–	–	–
Urine (diluted)	–	0.05	–	–
Standard	–	–	0.05	–
Purified water	–	–	–	0.05
Mix all tubes and incubate 1 – 5 minutes at ambient temperature, then add				
Picric acid	0.2	0.2	0.2	0.2

Mix and after exactly 30 seconds measure at wavelength 500 nm against purified water the initial absorbances of blank (A_0), serum (A_{serum}), urine (A_{urine}) and standard (A_{st}) (1st measurement). Exactly 90 seconds after 1st measurement read the final absorbances (2nd measurement) of blank, serum and urine samples, and the standard.

Evaluation:

Calculate the differences of absorbances between 2nd and 1st measurements:

$$\mathbf{\Delta A = A2 - A1}$$

Subtract the ΔA of the blank from the ΔA of serum, urine and standard.

Concentration of creatinine in serum (S-Creatinine):

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

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

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

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

$$\text{dU-Creatinine (mmol/24 hrs)} = \text{U-Creatinine (mmol/l)} \times \text{Volume of urine (liters/24 hrs)}$$

Task 2: Calculation of clearance of endogenous creatinine

Clearance of endogenous creatinine (Cl_{Cr}):

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

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:

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

Estimation of Cl_{Cr} for women:

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

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 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	-
Purified 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 550 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)

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 4: Quantitative estimation of protein in urine and CSF

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. Urine – unknown sample (infectious material)
5. Cerebrospinal fluid – unknown sample (infectious material)

Procedure:

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

Measure in ml:	Urine sample (Tube No. 1)	CSF sample (Tube No. 2)	Standard (Tube No. 3)	Blank (Tube No. 4)
Urine sample	0.02	–	–	–
CSF sample	–	0.02	–	–
Standard	–	–	0.02	–
Purified water	–	–	–	0.02
Buffer	0.5	0.5	0.5	0.5
Chromogen	0.5	0.5	0.5	0.5
Mix the tubes and incubate 5 minutes at the room temperature. Measure the absorbances of sample and standard against the blank in 1 cm cuvette at 600 nm.				

Calculations:

$$\text{Total protein (g/l)} = \frac{A_{Urine}}{A_{Standard}} \times c_{Standard}\ (g/l)$$

$$\text{Total protein (g/l)} = \frac{A_{CSF}}{A_{Standard}} \times c_{Standard}\ (g/l)$$

Task 5: Evaluation of electrophoresis of urinary proteins

Procedure and evaluation:

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.

Task 6: Evaluation of isoelectrophoreograms of CSF and serum

Procedure and evaluation:

Make a sketch of isoelectrophoreograms of cerebrospinal fluid and serum from two patients. Try to determine which of the possible five types these results represent and include an example of a relevant pathological condition.

Task 7: Potentiometric estimation of Na^+ in urine

Reagents:

1. Dilution buffer: 1.98 g of acetic acid 100 % and 4.09 g of ethanolamine in 1000 ml, pH 9.9.
2. Standard NaCl solutions **0.1 mol/l**, **0.01 mol/l**, and **0.001 mol/l** in the dilution buffer.
3. Urine sample of unknown Na^+ concentration.

Procedure:

a. Dilute the urine sample 10× with the buffer: mix 5 ml of sample with 45 ml of buffer.

b. Follow the provided instructions for use of pH-meter as a mV-meter and measure the electrode potentials in the calibration solutions of NaCl 1 – 3 as well as in the diluted urine sample. Do not rinse the electrode with distilled water in between measurements; wipe it gently (avoid rubbing!) instead. Record all measured values of E (mV) into table in your report.

c. Create a calibration graph from the measured values of the electrode potentials and the corresponding pNa values of the standard solutions. Plot the pNa values on the x axis and the potential in mV on the y axis. Next, use the calibration graph to read the pNa for the analyzed urine sample.

d. Convert the pNa value of diluted urine sample into Na^+ concentration:

$$\mathbf{pNa = -\log[Na^+]} \qquad \mathbf{[Na^+]_{diluted\ urine} = 10^{-pNa}}$$

e. Correct for dilution of urine:

$$\mathbf{U\text{-}Na^+ = 10 \times [Na^+]_{diluted\ urine}}$$

Calculations:

(Use the values from Task 1 and 2)

Daily output of Na^+ into urine:

dU- Na^+ = U-Na^+ (mol/l) × Vol. urine (liters/24 hrs)

Fractional excretion (FE) of Na^+:

U_{Cr} Concentration of creatinine in urine (mmol/l): .....

P_{Cr} Concentration of creatinine in serum (mmol/l): .....

U_{Na} Concentration of sodium in urine (mmol/l): .....

P_{Na} Concentration of sodium in serum (mmol/l): .....

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

Tubular resorption (TR) of Na^+:

$$TR_{Na} = 1 - FE_{Na}$$