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

Lab report from the practical lesson on biochemistry

Topic: Metabolism of calcium and phosphate. Osteoporosis

Task 1: Estimation of inorganic phosphate in serum and urine

Principle:

Results:

	Serum sample (Tube No. 1)	Urine sample (Tube No. 2)	Standard (Tube No. 3)	Blank (Tube No. 4)
A 340 nm				-

Calculations:

Concentration of inorganic phosphate in serum (fS-P inorg.):

$$\text{fS-Inorganic phosphate (mmol/l)} = \frac{A_{serum}}{A_{standard}} \times c_{standard}$$

$$\text{fS-Inorganic phosphate (mmol/l)} = \frac{\ldots\ldots}{\ldots\ldots} \times \ldots\ldots = \ldots\ldots$$

Concentration of inorganic phosphate in urine (U-P inorg.):

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

$$\text{U- Inorganic phosphate (mmol/l)} = \frac{\ldots\ldots}{\ldots\ldots} \times \ldots\ldots \times \ldots\ldots = \ldots\ldots$$

Daily output of inorganic phosphate into urine (dU-P):

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

dU-Inorg. phosphate (mmol/24 hrs) = …….. × …….. = …….

Conclusion:
Evaluate both serum phosphate and daily output of phosphate to urine.

Task 2: Estimation of total calcium in serum and urine

Principle:

Results:

	Serum sample (Tube No. 1)	Urine sample (Tube No. 2)	Standard (Tube No. 3)	Blank (Tube No. 4)
A 650 nm				-

Calculations:

Concentration of total calcium in the serum (S-Ca):

$$\text{S-Calcium (mmol/l)} = \frac{A_{serum}}{A_{standard}} \times c_{standard}$$

$$\text{S-Calcium (mmol/l)} = \frac{\ldots\ldots\ldots}{\ldots\ldots\ldots} \times \ldots\ldots\ldots = \ldots\ldots\ldots\ldots\ldots$$

Concentration of calcium in the urine (U-Calcium):

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

$$\text{U- Calcium (mmol/l)} = \frac{\ldots\ldots\ldots}{\ldots\ldots\ldots} \times \ldots\ldots\ldots \times \ldots\ldots\ldots = \ldots\ldots\ldots\ldots\ldots$$

Daily output of calcium into urine (dU-Calcium):

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

dU-Calcium (mmol/24 hrs) = …….. × …….. = …….

Conclusion:
Evaluate both serum calcium and daily output of calcium to urine.

Task 3: Estimation of catalytic concentration of alkaline phosphatase and its isoforms

Principle:
What is chemistry of the ALP estimation? How is separate measurement of different isoenzymes achieved?

A. End-point assay with estimation of isoforms

Results and calculations:

	Native serum sample (S1)	Control for native sample (S2)	Heat-inactivated sample (T1)	Control for heat-inactivated (T2)
A 420 nm				

Subtraction of control absorbances:

$\Delta A_S = A_{S1} - A_{S2}$

ΔA_S = ... − ..... = ...

$\Delta A_T = A_{T1} - A_{T2}$

ΔA_T = ... − ..... = ...

Total catalytic concentration of ALP:

Total ALP (µkat/l) = $\Delta A_S \times 10.263$ = ... × 10.263 = ...

Catalytic concentration of the liver isoenzyme:

Liver isoenzyme ALP (µkat/l) = $1.5 \times \Delta A_T \times 10.263$ = ...

Catalytic concentration of the bone isoenzyme:

Bone isoenzyme ALP (µkat/l) = Total ALP (µkat/l) − Liver isoenzyme ALP (µkat/l)

Bone isoenzyme ALP (µkat/l) = ... − ..... = ...

B. Total ALP – kinetic assay

Results and calculations:

Measured absorbances:	Difference per minute:
A_0 ……	
A_1 ……	$\Delta A_1 = A_1 - A_0$ = …….
A_2 ……	$\Delta A_2 = A_2 - A_1$ = …….
A_3 ……	$\Delta A_3 = A_3 - A_2$ = …….
A_4 ……	$\Delta A_4 = A_4 - A_3$ = …….
A_5 ……	$\Delta A_5 = A_5 - A_4$ = …….

Mean difference of absorbance per minute ΔA420/min.:

Catalytic concentration ALP (µkat/l) = ΔA420/min. × 72.8 = …….

Conclusion:
Evaluate both total ALP and the proportion of both isoforms.

Task 4: Solubility of various calcium salts

Principle:

A. Solubility of calcium salts in water and HCl

Results:

	Tube No. 1 $CaCl_2$	**Tube No. 2** $CaCO_3$	**Tube No. 3** $Ca_3(PO_4)_2$	**Tube No. 4** $Ca_3(PO_4)_2$ + Na_2EDTA
Solubility in water				
Solubility in HCl	–			
Solubility in $NaHCO_3$	–	–		

B. Influence of some food components on solubility of calcium salts

Results:

	Tube No. 1 $CaCl_2$ + Ammonium oxalate	**Tube No. 2** $CaCl_2$ + Lactose
Result		

Conclusion: