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

Lab report form for the practical lesson on biochemistry

Topic: **Amino acids – reactions and conversions. Nitrogen balance**

Task 1: Colored reactions of amino acids and proteins

1.1 Ninhydrin reaction

Principle:
What is ninhydrin? Which substances react?

Results:

Sample:	**1** Alanine	**2** Proline	**4** Egg albumin	**5** Gelatin
Color change:				
Evaluation:				

Conclusion:

1.2 Xanthoproteic reaction

Principle:
Use structural formulas. What substances react?

Results:

Sample:	**1** Alanine	**2** Tyrosine	**3** Egg albumin	**4** Gelatin
Color change:				
Evaluation:				

Conclusion:

1.3 Reaction of cysteine – proof of sulfur in protein molecules

Principle:

Results:

Sample:	**1** Alanine	**2** Cysteine	**3** Egg albumin	**4** Gelatin
Color change:				
Evaluation:				

Conclusion:

1.4 Biuret reaction

Principle:
What is biuret? What is biuret reaction? Which substances react?

Results:

Sample:	**1** Biuret	**2** Alanine	**3** Egg albumin	**4** Gelatin
Color change:				
Evaluation:				

Conclusion:

Task 2: Estimation of aspartate aminotransferase (AST) in serum

Principle:
Use structural formulas and include also the indication reaction.

Results and calculations:

Time:		ΔA_{340}
0	A_0 ……	
1 minute	A_1 ……	$A_0 - A_1 \rightarrow \Delta A_1$ ……
2 minutes	A_2 ……	$A_1 - A_2 \rightarrow \Delta A_2$ ……
3 minutes	A_3 ……	$A_2 - A_3 \rightarrow \Delta A_3$ ……

$$\Delta A_{340} = \frac{\Delta A_1 + \Delta A_2 + \Delta A_3}{3} = \text{……..}$$

S-AST (µkat/l) = ΔA_{340}/min. × 36.2 = ……..

Conclusion:
Compare the catalytic concentration of AST in your sample with reference values.

Task 3: Estimation of alanine aminotransferase (ALT) in serum

Principle:
Use structural formulas and include also the indication reaction.

Results and calculations:

Time:		ΔA_{340}
0	A_0 ……	
1 minute	A_1 ……	$A_0 - A_1 \rightarrow \Delta A_1$ ……
2 minutes	A_2 ……	$A_1 - A_2 \rightarrow \Delta A_2$ ……
3 minutes	A_3 ……	$A_2 - A_3 \rightarrow \Delta A_3$ ……
4 minutes	A_4 ……	$A_3 - A_4 \rightarrow \Delta A_4$ ……
5 minutes	A_5 ……	$A_4 - A_5 \rightarrow \Delta A_5$ ……

$$\Delta A_{340} = \frac{\Delta A_1 + \Delta A_2 + \Delta A_3 + \Delta A_4 + \Delta A_5}{5} = \text{……..}$$

S-ALT (µkat/l) = ΔA_{340}/min. × 29.5 = ……..

Conclusion:
Compare the catalytic concentration of ALT in your sample with reference values.

Task 4: Estimation of urea in serum and urine

Principle:

Results:

	Cuvette No. 1 Serum sample	**Cuvette No. 2** Urine sample	**Cuvette No. 3** Standard	**Cuvette No. 4** Reagent blank
Absorbance A_1				
Absorbance A_2				

Calculations:

Concentration of urea in serum (S-Urea):

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

S-Urea (mmol/l) = ——————————— × ……. = ……

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

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

U-Urea (mmol/l) = ——————————— × ……. × …..…… = ……

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

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

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

Conclusion:
Compare the urea in serum and urine with reference values.

Task 5: Calculation of nitrogen balance

Principle:
What is nitrogen balance?

Summary of data:

Calculations:

Intake of nitrogen (g/24 hours) = Intake of proteins/amino acids × 0.16

Intake of nitrogen (g/24 hours) = ……

Catabolic nitrogen (g/24 hrs) = Urea in urine (mmol/L) × V (L) × 0.028 × 1.2 + Δ Urea in serum (mmol/l) × 0.028 × Body weight (kg) × Factor of body water + Loss of urea by stool and skin

Catabolic nitrogen (g/24 hrs) = …….

Nitrogen Balance = Intake of Nitrogen (g/24 hrs) − Catabolic Nitrogen (g/24 hrs)

Nitrogen balance = …… − …….. = …….....

Conclusion: