Comenius University in Bratislava, Faculty of Medicine
Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry

LABORATORY PROTOCOL SS - 12th seminar

Determination of enzymatic activity of alkaline (ALP) phosphatase

Name, study group:	Date:

Alkaline phosphatase (ALP) is present in very different tissues including liver, bone, intestine and placenta. Increased activity of serum ALP is associated with two groups of diseases – hepatobiliary diseases and bone diseases. Activity of ALP is increased in all types of cholestasis (disruption of drainage of bile from liver to small intestine), especially in obstructive jaundice. ALP is also increased in bone diseases characterized by hyperactivity of osteoblasts and increased remodelation of bone, such as Paget´s disease, hyperparathyroidism, osteomalacia, bone fractures and malign bone tumours. Significant increase of ALP activity is also present in children and adolescents, that are undergoing period of rapid growth.

Principle:
ALP catalyses hydrolysis of phosphoester bond, which is present in the molecule of nitrophenyl phosphate (colourless) in alkaline environment. Products of this reaction are free phosphate and nitrophenol, which is also of colourless. Adding NaOH stops the reaction and creates yellow product – nitrophenolate which is suitable for spectrophotometric determination.

Procedure:

	ALP	reference sample
substrate (pH 10)	0.2 mL	0.2 mL
serum ALP (1:10)	0.2 mL	---
physiological solution	---	0.2 mL
Let the samples stand for 15 minutes.		
0.05 mol/L NaOH	1 mL	1 mL
Mix the samples and measure absorbance at 440 nm.		

Calculation:

	ALP
absorbance	
substrate (nmol)	
nmol/L	
nkat/L	
correction for dilution	
μkat/L	

Reference values - ALP

Adults	men	0,67 – 2,15 μkat/L
	women	0,58 – 1,74 μkat/L

Conclusion:

Determination of enzymatic activity of acidic (ACP) phosphatase

Acidic phosphatase (ACP) cleaves phosphate from compounds in acidic environment. Men have higher values of ACP than women. Highest activities are found in cells of prostate, osteoclasts, kidney tissue, erythrocytes and thrombocytes.
Serum activity of ACP increases in prostatic cancer, increased bone resorption, kidney disorders, hemolytic anemia and thrombosis.

ACP has two isoenzymes:

- tartrate unstable isoenzyme (prostatic)
- tartrate stable isoenzyme (present in bones, erythrocytes, thrombocytes)

In laboratory, total activity of ACP is measured first. Then prostatic isoenzyme is inactivated by sodium tartrate and the activity of tartrate stable isoenzyme is measured. Finally, activity of prostatic isoenzyme is calculated:

Prostatic ACP = total ACP - tartrate stable ACP

Principle:
ACP catalyses hydrolysis of phosphoester bond, which is present in the molecule of nitrophenyl phosphate (colourless) in acidic environment. Products of this reaction are free phosphate and nitrophenol, which is also of colourless. Adding NaOH stops the reaction and creates yellow product – nitrophenolate which is suitable for spectrophotometric determination.

Procedure:

	ACP	reference sample
substrate (pH 5.5)	0.2 mL	0.2 mL
serum 1 (1:2)	0.2 mL	---
saline	---	0.2 mL
Let the samples stand for 15 minutes.		
0.05 mol/L NaOH	1 mL	1 mL
Mix the samples and measure absorbance at 440 nm.		

Calculation:

	ACP
absorbance	
substrate (nmol)	
nmol/L	
correction for dilution	
nkat/L	

Reference values - ACP

Adults	total ACP	up to 110 nkat/L
	prostatic ACP	up to 58 nkat/L

Conclusion: