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LABORATORY PROTOCOL SS12

Determination of enzymatic activity of alkaline phosphatase (ALP)

Name, group:	Date:

Alkaline phosphatase (ALP) is present in very different tissues including liver, bone, intestine and placenta. We are interested in serum activity of ALP in two groups of diseases – hepatobiliary diseases and bone diseases, in which there is an increase of osteoblast activity. Activity of ALP is increased in all types of cholestase (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 catalyzes hydrolysis of phosphoesteric bond, which is present in the molecule of nitrophenylphosphate (non-colored). Products of this reaction is free phosphate and nitrophenol, which is also of no color. Adding NaOH stops the reaction and creates yellow product – nitrophenolate which is suitable for spectrophotometrical determination.

Procedure:

	S-ALP	reference sample
substrate	0,2 ml	0,2 ml
serum 1 (1:10)	0,2 ml	---
serum 2 (1:10)	---	---
saline	---	0,2 ml
We let the samples stand for 15 minutes.		
0,05 mol/l NaOH	1 ml	1 ml
We mix the samples and measure absorbance at 440 nm.		

Calculation:

	Patient 1
	S-ALP
absorbance	
substrate (nmol)	
nmol/l	
ncat/l	
μcat/l	

Reference values

Children	up to 1 year	up to 7,69 μcat/l
	7 - 12 years	up to 5 μcat/l
Adults	men	0,67 – 2,15 μcat/l
	women	0,58 – 1,74 μcat/l

Conclusion:

Determination of enzymatic activity of acidic phosphatase (ACP)

Acidic phosphatase (ACP) cleaves phosphate from compounds in acidic environment. It is measured in blood plasma in ncat/l. Men have higher values of ACP than women. Highest activities can be found in cells of prostate, osteoclasts, kidney tissue, erythrocytes and thrombocytes.
Plasmatic activity of ACP increases in cases of prostatic cancer, increased bone resorption, kidney disorders, hemolytic anemias and thrombosis.

Acidis phosphatase has two isoenzymes:
– tartarate unstable isoenzyme (prostatic)
– tartarate stable isoenzyme

In laboratory total activity of ACP is measured first. Then prostatic isoenzyme is inactivated by sodium tartarate and we measure activity of tartarate stable isoenzyme. Finally we calculate activity of prostatic isoenzyme:
Prostatic ACP = total ACP minus tartarate stable ACP

Procedure:

	S-ACP	reference sample
substrate	0,2 ml	0,2 ml
serum 1 (1:2)	0,2 ml	---
saline	---	0,2 ml
We let the samples stand for 15 minutes.		
0,05 mol/l NaOH	1 ml	1 ml
We mix the samples and measure absorbance at 440 nm.		

Calculation:

	Patient 1
	S-ACP
absorbance	
substrate (nmol)	
nmol/l	
ncat/l	

Reference values

Adults	total ACP	up to 110 ncat/l
	prostatic ACP	up to 58 ncat/l

Conclusion: