

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

LABORATORY PROTOCOL GM-WS - 10th seminar

Determination of activities of transaminases (AST and ALT)

Name, group:	Date:
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Principle:

Oxaloacetate is a product of the reaction catalyzed by aspartate aminotransferase (AST) and is very quickly spontaneously decarboxylated to pyruvate. Pyruvate is also the product of the reaction catalyzed by alanine aminotransferase (ALT). Activities of aminotransferases are determined by measuring of amount of hydrazone (compound, which is a product of a reaction of pyruvate with 2,4-dinitrophenylhydrazine). Addition of NaOH creates sodium salt of pyruvate hydrazone, which is of brown color and the intensity of color is proportional to amount of pyruvate and therefore it is proportional to activities of ALT and AST.

Procedure:

	ALT			AST		
	ALT ₁	ALT ₂	ref. for ALT	AST ₁	AST ₂	ref. for AST
ALT substrate	0.5 ml	0.5 ml	0.5 ml	---	---	---
AST substrate	---	---	---	0.5 ml	0.5 ml	0.5 ml
serum 1	0.1 ml	---	---	0.1 ml	---	---
serum 2 (dilution 1:10)	---	0.1 ml	---	---	0.1 ml	---
physiol. solution	---	---	0.1 ml	---	---	0.1 ml
We let the samples stand for 20 minutes.						
reagent	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
We mix the samples and let them stand for 10 minutes.						
NaOH	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml
We mix the samples and let them stand for 5 minutes and measure absorbance at 505 nm.						

Calculation:

	Patient 1		Patient 2	
	ALT ₁	AST ₁	ALT ₂	AST ₂
absorbance				
nmol of pyruvate/0.1 ml				
nmol of pyruvate/l				
correction for dilution	---	---		
activity (nkat/l)				
activity (μkat/l)				

Reference values:

	ALT		AST	
	μkat/l	U/l	μkat/l	U/l
men	0.08 – 0.38	5 – 19	0.08 – 0.28	5 – 17
women	0.08 – 0.32	5 – 17	0.08 – 0.25	5 – 15
newborns	< 0.46	< 28	< 0.58	< 35

Conclusion:

Literature:

Detoxification of ammonia – urea cycle, inter-organ relationships
Lippincott's: Chapter 19, Part V - VII.