BCH 471

Determination of non functional plasma enzymes

Determination of ALT in serum

Objectives

- To determine the level of ALT in serum.
- To evaluate the presence of tissue damage.



Differences of Functional and Nonfunctional plasma enzymes

	Functional plasma enzymes	Nonfunctional plasma enzymes	
Their substrate	Always present in the blood	Absent from the blood	
Site of synthesis	Liver	Different organs e.g. liver, heart, muscles, and brain	
Effect of diseases	Decrease in liver diseases	increase in different organ diseases	
Examples	Clotting factors Lipoprotein Lipase	ALT LDH Acid Phosphatase Amylase	

Sources of Nonfunctional Plasma Enzyme



Measurement of Non Functional Enzymes is Important for 2 Medical Reasons:

• Diagnosis of diseases

 Prognosis of the disease: following up of the treatment by measuring plasma enzymes before and after treatment.

Alanine transaminase (ALT)

- ALT is an enzyme that catalyzes a type of reaction (transamination) between an amino acid and α-keto acid.
- It is important in the production of various amino acids.



ALT diagnostic importance

- ALT is found in serum (at low level) but is most commonly is associated with the <u>liver</u>.
- thus, an elevated level ALT is a <u>sensitive index of acute</u> <u>hepatocellular injury.</u>
- Elevated serum ALT level are found in hepatitis, cirrhosis, and obstructive jaundice.



• NORMAL RANGE OF ALT:

(up to 42) U/L \rightarrow males (up to 32) U/L \rightarrow females

Principle

1- ALT *"present in serum sample"* catalyzes the transfer of an amino group from alanine to α -ketoglutarate in the following reaction:

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Alanine + \alpha- ketoglutarate \rightarrow Pyruvate + glutamate
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2- Then, the pyruvate formed in the reaction is reduced to L-Lactate by Lactate dehydrogenase (LDH) *"found in ALT reagent".*

Pyruvate + NADH+H⁺ \rightarrow L-Lactate+ NAD⁺ +H₂O

3- The absorbance at 340nm is measured each minute, resulting in <u>decreased</u> readings due to the oxidation of NADH

Principle





Pipette into clean and dry test tubes:

ALT Reagent	1 ml			
Pre-warm at 37°C for 3 minutes and add				
Serum Sample	0.1 ml	ml→ µl (x 1000)		
Mix and incubated at 37 °C for 1 minute, then read absorbance (at				
340 nm against distilled water) every minute for 3 minutes) and				
determine ∆A/min				

Choose the following on the spectrophotometer:

2) Applications \rightarrow 2) Simple Kinetics \rightarrow wave length (340 nm) \rightarrow 1) Seconds \rightarrow Duration (120 sec =2min) \rightarrow Intervals (60 sec= 1 min) \rightarrow Print Data Table (off) \rightarrow Press start (2 times)



Time	Absorbance 340nm		∆A/min=((A1-A2)+(A2-A3))/2
1 min	A1		
2 min	A2		
3 min	A 3		

Calculations

ALT Activity (U/L) = Δ A/min x 1768 ALT Activity (U/L) =

Question

Discuss the diagnostic significance of Creatine Kinase and Troponin I, and determine which marker is most sensitive and specific for cardiac damage?