

Determination of plasma enzymes

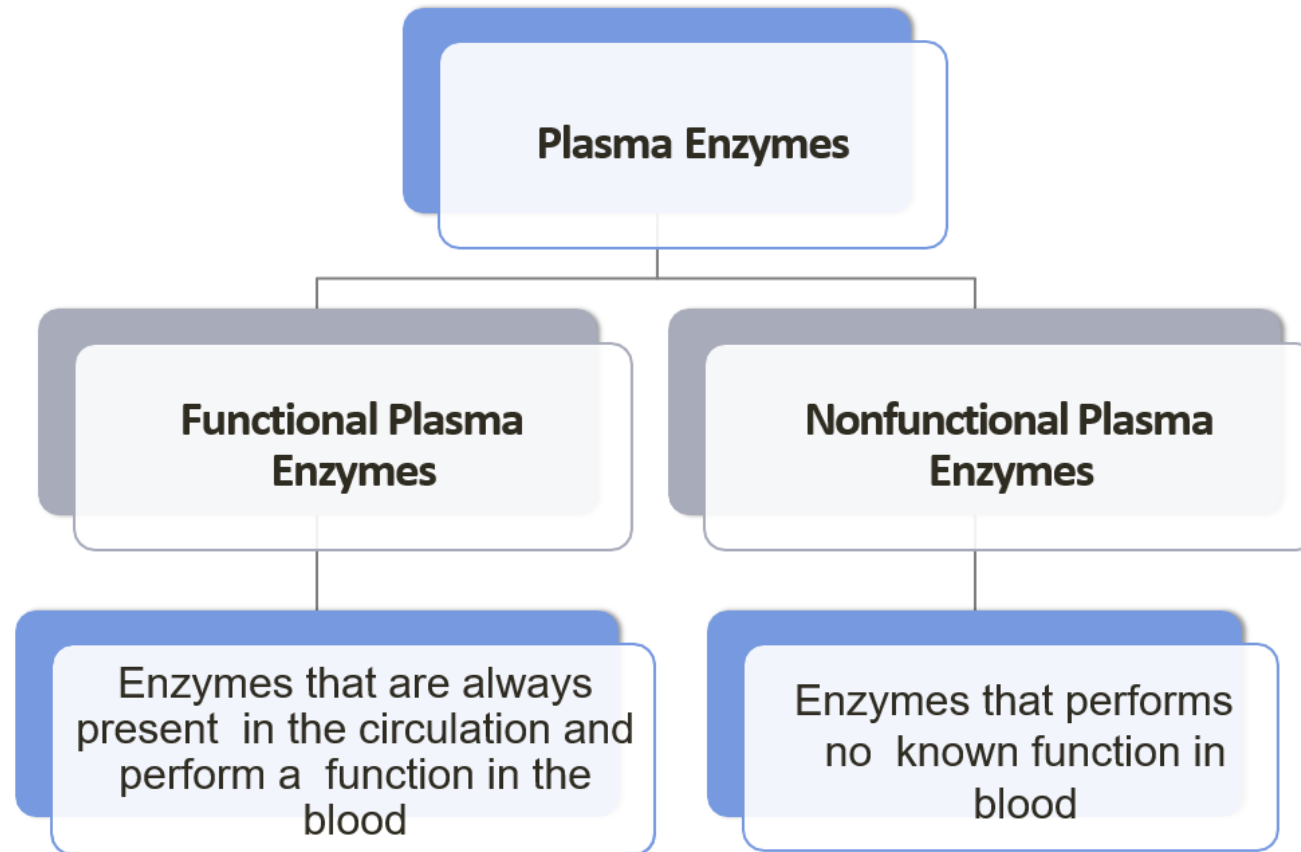
Determination of ALT in serum

BCH 471

[Practical]



Most clinical enzyme measurements using serum or plasma, occasionally other fluids, such as urine and gut secretions, are investigated.

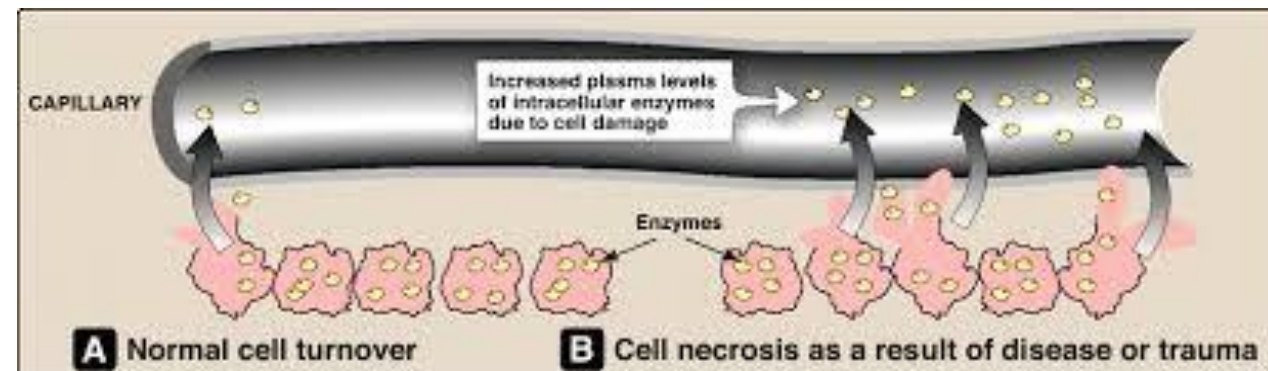


Differences of Functional and Nonfunctional plasma enzymes

	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	Different enzymes increase in different organ diseases
Examples	Clotting factors Lipoprotein Lipase	ALT LDH Acid Phosphatase Amylase

Sources of Nonfunctional Plasma Enzyme

- Cell damage with the release of its content of enzymes into blood e.g. Myocardial infarction and viral hepatitis
- Obstruction of normal pathways e.g. Obstruction of bile duct increases alkaline phosphatase
- Increase of the enzyme synthesis e.g. bilirubin increases the rate of synthesis of alkaline phosphatase in obstructive liver disease
- Increased permeability of cell membrane as in hypoxia



Medical Importance of Non Functional Plasma Enzymes

- Measurement of non functional enzymes is important for:
- Diagnosis of diseases
- Prognosis of the disease: following up of the treatment by measuring plasma enzymes before and after treatment.



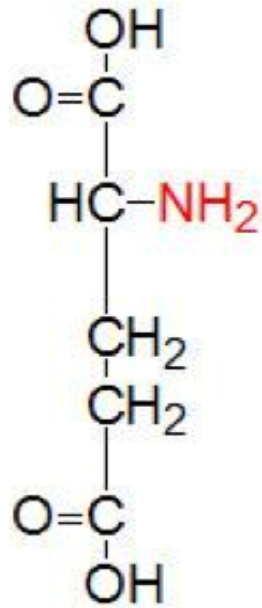
Alanine transaminase

- 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.



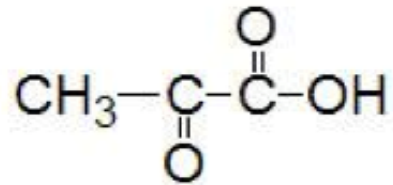
ALT Reaction

transamination reaction involves removing the amino group from the amino acid, leaving behind an α -keto acid, and transferring it to the reactant α -keto acid and converting it into an amino acid.



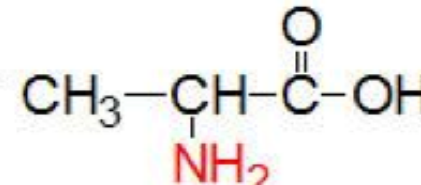
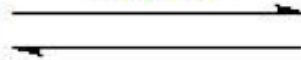
glutamate

+



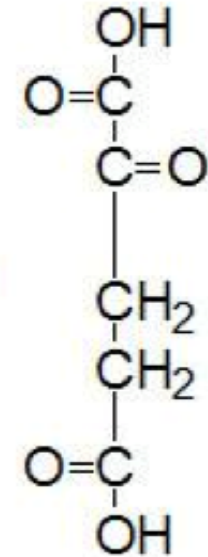
pyruvate

ALT



alanine

+



α -ketoglutarate

It is also called: Serum glutamic pyruvic transaminase (SGPT) or Alanine aminotransferase (ALT).

ALT diagnostic importance

- ALT is found in serum (at low level) but is most commonly associated with the liver.
- thus, an **elevated level ALT** is a sensitive index of acute hepatocellular injury.
- Elevated serum ALT (SGPT) level are found in **hepatitis, cirrhosis** , and obstructive jaundice. Levels of ALT are only slightly elevated in patient following a myocardial infraction.



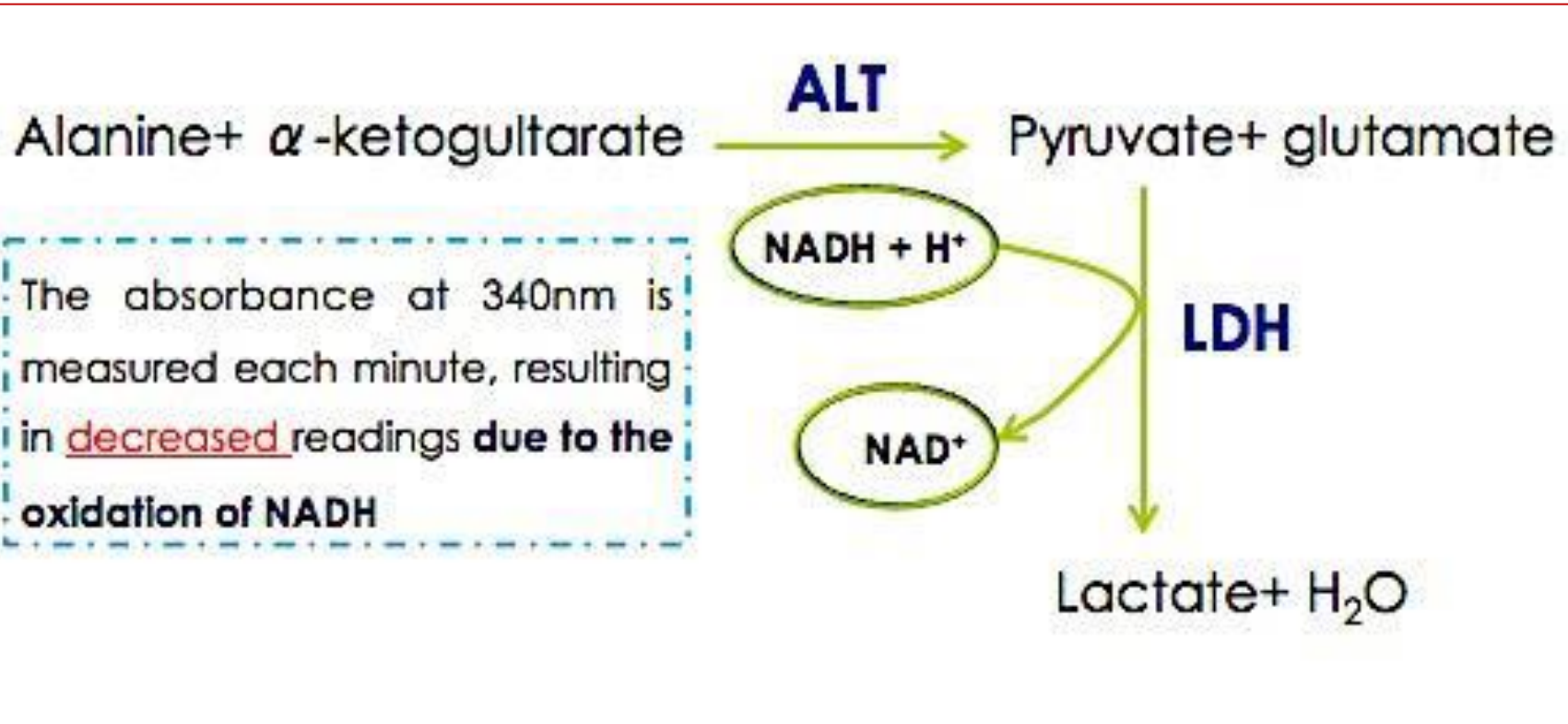
Practical Part

ALT Assay

Objectives:

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

Principle



Method

	Tube
ALT reagent	3 ml
Pre-warm at 37 °C for 3 minutes and add	
Sample (serum)	0.2ml (μ l)?
Mix and incubate at 37 °C for 1 minutes, then read the absorbance at 340 nm against <u>distilled water (blank)</u> every minute for 3 minutes and determine $\Delta A/\text{min}$.	

2) Applications → 2) Simple Kinetics → wave length (340 nm) → 1) Seconds → Duration (180 sec = 3 min) → Intervals (60 sec= 1 min) → Print Data Table (off) → Press start (2 times)

Results

	Time (min)	Absorbance at 340 nm
A1	1	
A2	2	
A3	3	

Calculations

$$\Delta A_1 = A_1 - A_2$$

$$\Delta A_2 = A_2 - A_3$$

$$\Delta A/\text{min} = (\Delta A_1 + \Delta A_2) / 2$$

$$\underline{\text{ALT (U/L)}} = \Delta A/\text{min} \times 1768$$

NORMAL RANGE OF ALT: (up to 42) U/L males - (up to 32) U/L females