



Lab (2) Determination of Non-functional Plasma Enzymes in Serum



BCH 471 [Practical]
Blood Biochemistry

Objectives

- To determine the level of alanine transaminase (ALT) in serum.
- To evaluate the presence of tissue damage.

Blood Enzymes

- Plasma proteins, also termed **serum proteins** or **blood proteins**, are proteins present in blood plasma which have several functions.
- Some blood proteins also act as **enzymes**.
- **Clinical enzymology** deals with quantitative estimation of enzymes in body fluids in normal and disease conditions.
- Most clinical enzyme measurements using **serum** or **plasma**, occasionally other fluids, such as urine and gut secretions are also investigated.
- The most commonly used body fluid for this purpose is **SERUM**. (Why?)

Differences Between Plasma Enzymes



Plasma Enzymes

1. Plasma-specific Enzymes

Enzymes that are normally present in the plasma and perform their primary function in the blood.

2. Non-plasma-specific Enzymes

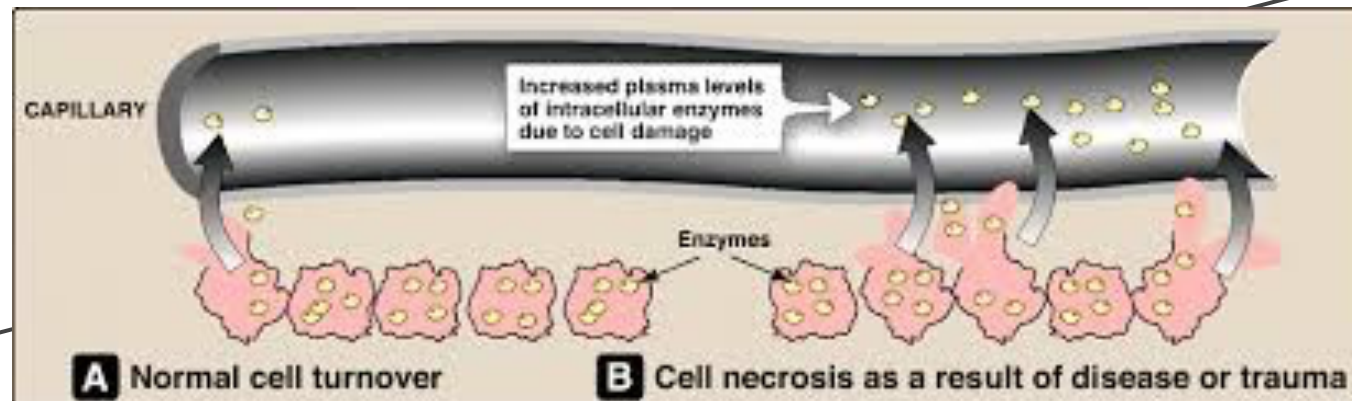
Intracellular enzymes that are normally present in very small amount in blood and perform no known function in blood.

	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	Thrombin Plasmin ceruplasmin	ALT LDH Acid Phosphatase Amylase

Sources of Nonfunctional Plasma Enzyme

1. **Cell damage** with the release of its content of enzymes into blood e.g. Myocardial infarction and viral hepatitis.
2. **Block in the secretory pathway** e.g. elevation of blood pancreatic amylase and lipase in pancreatitis.
3. **Increase enzyme synthesis** e.g. elevation of serum alkaline phosphatase in breast cancer.

So estimation of the plasma concentration of these enzymes in blood is useful for the diagnosis of disease depending on their tissue origin.



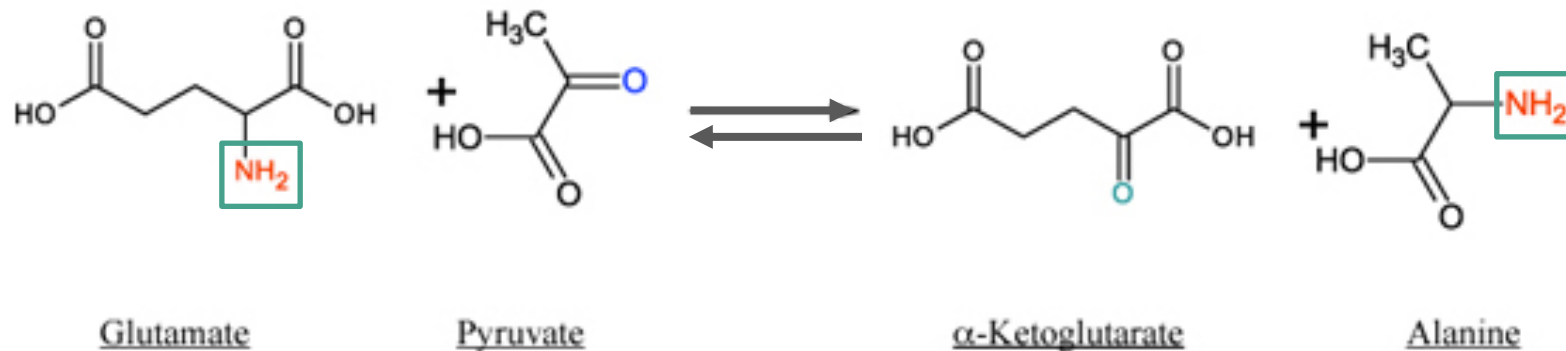
Clinical Significance of Non-Functional Plasma Enzymes

Measurement of non-functional enzymes is important for:

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

Alanine Transaminase

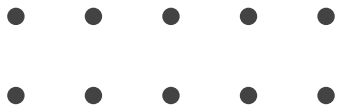
- ALT is an enzyme that catalyzes a type of reaction (**transamination**) between an amino acid and α -keto acid.
- It is important in the production of various amino acids.
- Also called alanine transferase (ALT), serum glutamate-pyruvate transaminase (SGPT).



- **Transamination reaction** is the process by which amino groups are removed from amino acids and transferred to acceptor keto-acids to generate the amino acid version of the keto-acid and the keto-acid version of the original amino acid.

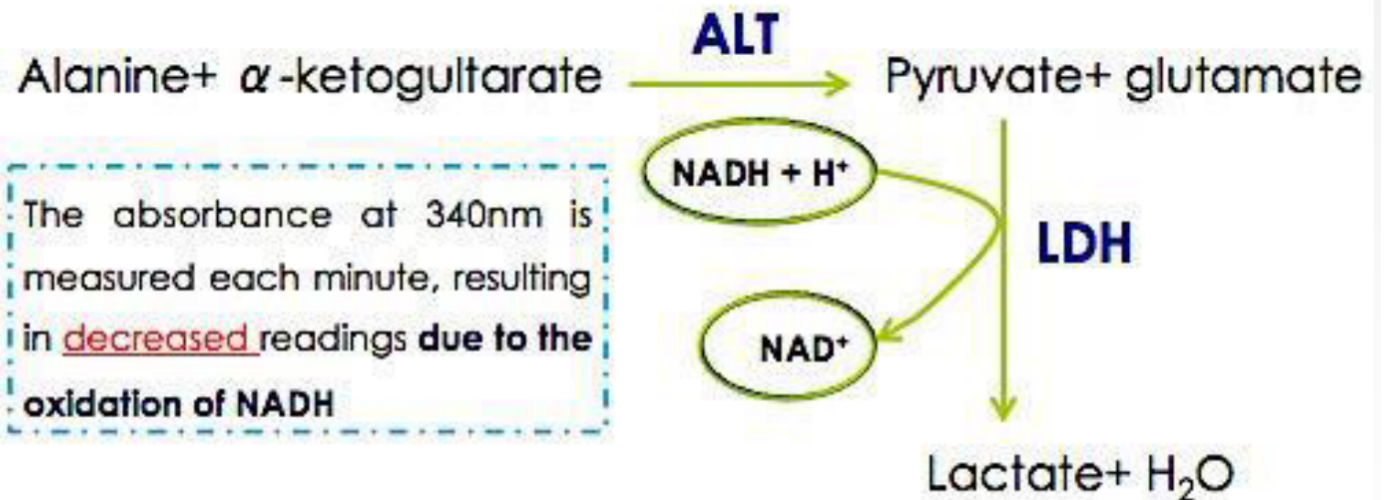
ALT Diagnostic Importance

- 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 infarction**.



Practical Part

Principle



Method

Tube	
ALT reagent	1 ml
Pre-warm at 37 °C for 3 minutes and add	
Sample (serum)	100 µl

Mix and incubate at 37 °C for 1 minutes, then read the absorbance at 340 nm against distilled water (blank) every minute for the next 2 minutes and determine $\Delta A/\text{min}$.

Measure enzyme kinetics using UV-visible spectroscopy:

2) Applications → 2) Simple Kinetics → wave length (340 nm) → 1) Seconds → Duration (120 sec = 2 min) →

Intervals (60 sec= 1 min) → Print Data Table (off) → Press start (2 times)

Results and Calculations

Results

Time (min)		Absorbance at 340 nm
A ₁	0	
A ₂	1	
A ₃	2	

Calculations

$$\Delta A_1 = A_1 - A_2 \quad \Delta A_2 = A_2 - A_3$$

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

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

Normal Values

Males: up to 42(U/L)

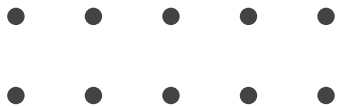
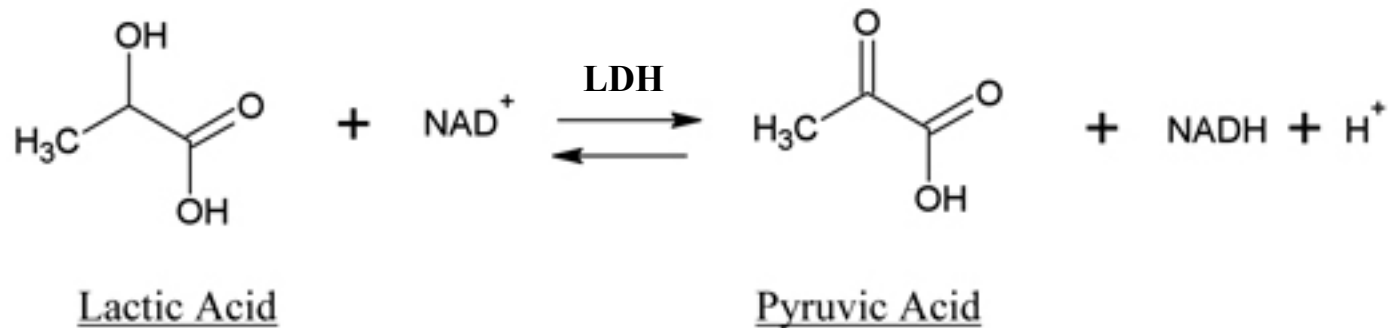
Female: up to 32(U/L)

Lactate Dehydrogenase (LDH)

- Lactic acid dehydrogenase (LDH) is an enzyme that helps produce energy.
- LDH is most often measured to evaluate the presence of tissue damage (diagnostic).
- The enzyme LDH is in many body tissues, especially the heart, liver, kidney, skeletal muscle, brain, blood cells and lungs.

LDH Reaction

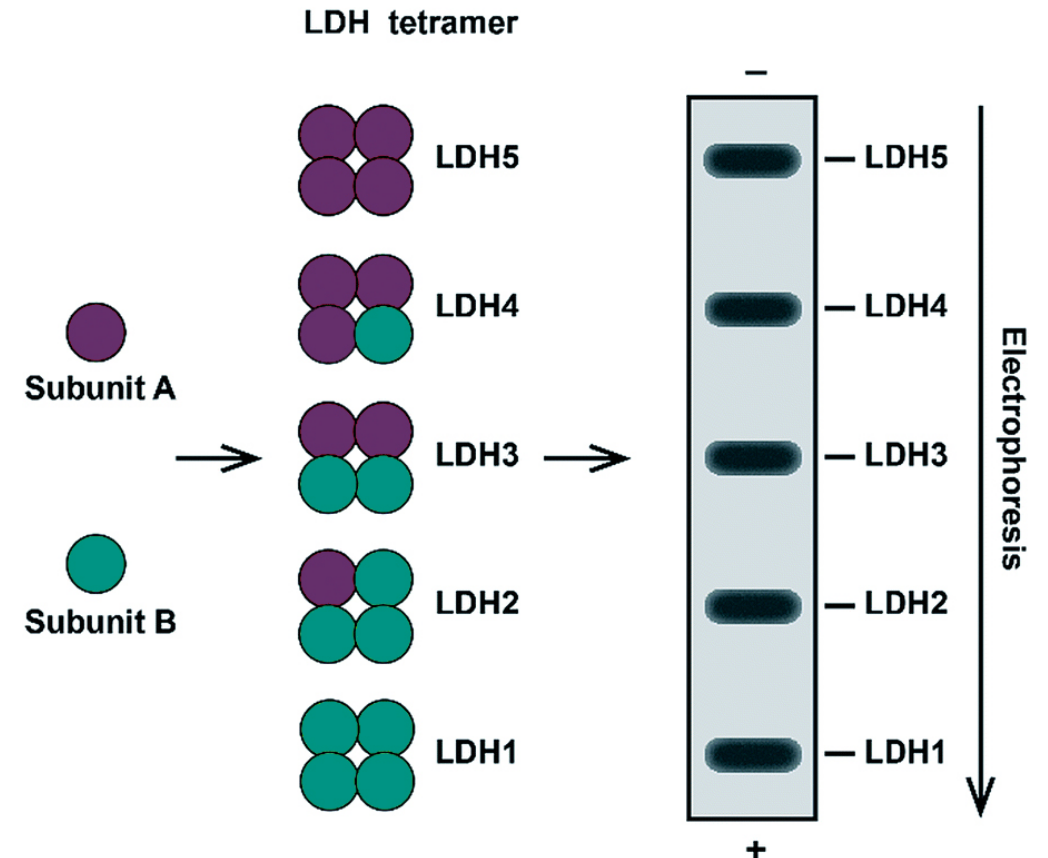
- LDH is a hydrogen transfer enzyme which catalyzes the **interconversion of pyruvate and lactate** with the mediation of NAD^+ as hydrogen acceptor, eventually converting pyruvate to glucose.
- The optimum pH for lactate pyruvate ($\text{L} \rightarrow \text{P}$) reaction is **8.8 – 9.8**, While for pyruvate to lactate ($\text{P} \rightarrow \text{L}$) is **7.7 – 7.8**.
- The enzyme is inhibited by **sulphydryl reagents** and **mercuric ions**.



LDH Isoenzymes

- LDH exists in **5 forms** (isoenzymes), which differ slightly in structure.
- All of these isoenzymes can be measured in the blood, and can be separated by electrophoresis.

Isoenzyme	Tissues	Diseases associated
LDH-1	Found primarily in heart muscle and RBC	Myocardial infarction
LDH-2	Highest in WBC , heart and RBC	○ Megaloblastic anemia ○ leukemia
LDH-3	Found in lung tissue	Pulmonary embolism
LDH-4	Highest in the kidney, placenta and pancreas	Pancreatitis
LDH-5	Highest in the liver and skeletal muscle	○ Toxic hepatitis with jaundice ○ Muscular dystrophy



LDH in plasma

Diseases

Examples

Myocardial infarction

Liver
Disease

Toxic jaundice

Viral hepatitis

Obstructive jaundice

Anemia

Pernicious anemia

Megaloblastic anemia

Renal Diseases

Tubular necrosis

Pyelonephritis

Malignant Disease

Lung Cancer

Hodgkin's disease