Blood Biochemistry BCH 220 [Practical]

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

# Objectives

- To determine the level of **lactate dehydrogenase (LDH)** in serum.
- To evaluate the presence of tissue damage.

# Blood Enzymes

- Plasma, serum or **blood proteins**, are proteins present in blood plasma which have several functions.
- Some blood proteins also act as **enzymes.**
- **Enzymes** are biocatalysts that increase the rate of the chemical reaction.
- **Clinical enzymology** refers to measurement of enzyme activity in body fluids for the diagnosis and treatment of diseases.
- 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 (Functional)**

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

2. **Non-plasma specific Enzymes (Non functional)**

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

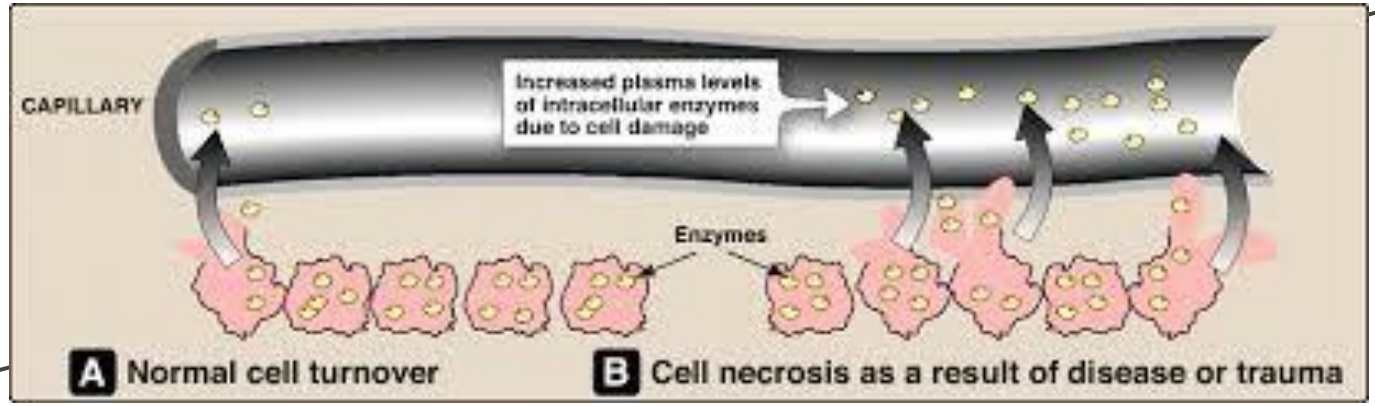
| | Functional plasma enzymes | Non functional plasma enzymes |
|---|---|---|
| **Their substrate** | Always present in the blood | Absent from the blood |
| **Site of synthesis** | Liver | Different organs |
| **Effect of diseases in its plasma levels** | Decrease in liver diseases | Different enzymes increase in different organ diseases |
| **Examples** | Thrombin<br>Plasmin | ALT<br>LDH |

**Pause and Think** **Which of these enzymes is a better diagnostic indicator ? Why?**

# Sources of Non functional Plasma Enzyme

1. **Cell damage** with the release of its content of enzymes into blood
2. **Block in the secretory pathway**
3. **Increase enzyme synthesis**

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:**

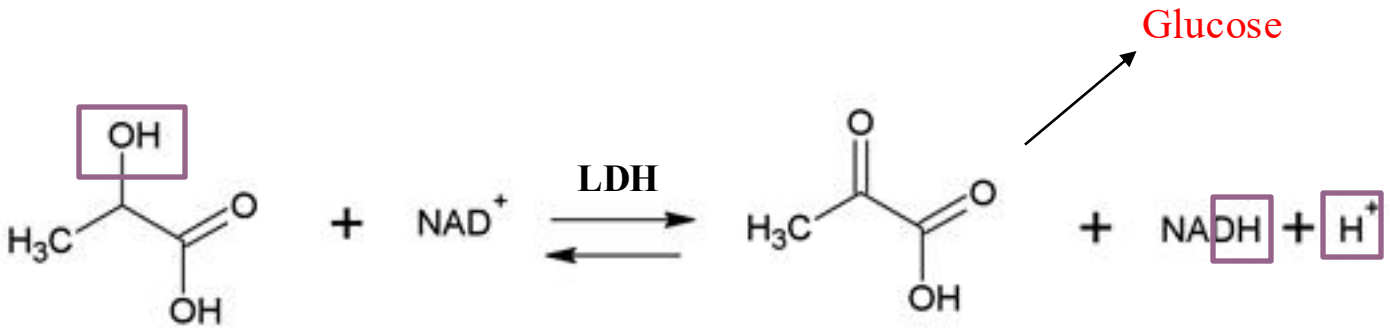
1. **Diagnosis of diseases.**
2. **Prognosis of the disease:** following up of the treatment by measuring plasma enzymes before and after treatment.

# Lactate Dehydrogenase (LDH)

- **Lactic acid dehydrogenase (LDH)** is an enzyme that helps produce energy.
- It is present in almost all of the tissues in the body and becomes elevated in response to cell damage
- 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 (L→ P) reaction is **8.8 – 9.8,** While for pyruvate to lactate (P→ L) is **7.7 – 7.8.**
- The enzyme is inhibited by **sulfhydryl reagents** and **mercuric ions.**




Lactic Acid Pyruvic Acid

# Practical Part

# Lactate Dehydrogenase Assay

## Principle

**LDH** catalysis the following reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{LDH} \text{L-Lactate} + NAD^+$$

- The rate of $NAD^+$ formation is indicated by **decrease in the absorbance at 340 nm** and it is directly proportional to serum LDH activity.

If:

- NADH is **reactant** → **decrease** the absorbance/min
- NADH is **product** → **increase** the absorbance/min

# Method

| | Tube |
|---|---|
| Sample (serum) | 10 µl |
| BUF (buffer/substrate) | 1000 µl |
| Mix and incubate **at 37°C for 1-5 minutes** | |
| SUB (substrate) | 250 µl |
| Mix, read the absorbance after **1 minute** and at the same time start the stop watch. Read the absorbance again exactly after **1, 2 and 3 minutes** | |

Measure enzyme kinetics using UV-visible spectroscopy:

**2) Applications → 2) Simple Kinetics → wave length (340 nm) → 1) Seconds → Duration (240 sec = 4 min) →**

**Intervals (60 sec= 1 min) → Print Data Table (off) → Press start (2 times) (*note: neglect the first reading*)**

# Results and Calculations

| | Time (min) | Absorbance at 340 nm |
|---|---|---|
| $A_1$ | 1 | |
| $A_2$ | 2 | |
| $A_3$ | 3 | |
| $A_4$ | 4 | |

**Calculations**

- $\mathbf{\Delta A_1 = A_1 - A_2}$ $\mathbf{\Delta A_2 = A_2 - A_3}$ $\mathbf{\Delta A_3 = A_3 - A_4}$
- $\Delta A/min = ( \Delta A_1 + \Delta A_2 + \mathbf{\Delta A_3} ) / 3$
- LDH(U/L) = **ΔA x 20000**

**Normal Values** **225 to 450 U/L Adults**