Blood Biochemistry BCH 471[Practical]

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

# 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.

# 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)**

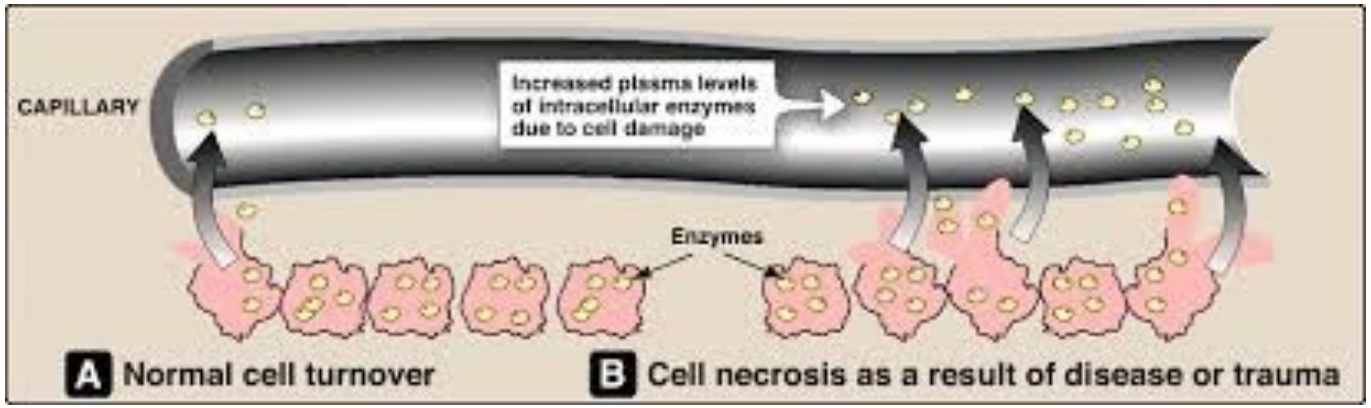
Intracellular enzymes that are normally present in very small amount in blood and perform no known function in blood.

| | Functional plasma enzymes | Non functional plasma enzymes |
|---|---|---|
| **Their substrate** | Always present in the blood | Absent from the blood |
| **Site of synthesis** | Liver | Different organs<br>e.g. liver, heart, muscles, and brain |
| **Effect of diseases in its plasma levels** | Decrease in liver diseases | Different enzymes increase in different organ diseases |
| **Examples** | Thrombin<br>Plasmin<br>Ceruplasmin | ALT<br>LDH<br>Acid Phosphatase<br>Amylase |

**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 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 **bone cancer**.
4. **Increased permeability of cell membrane** as in hypoxia.




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

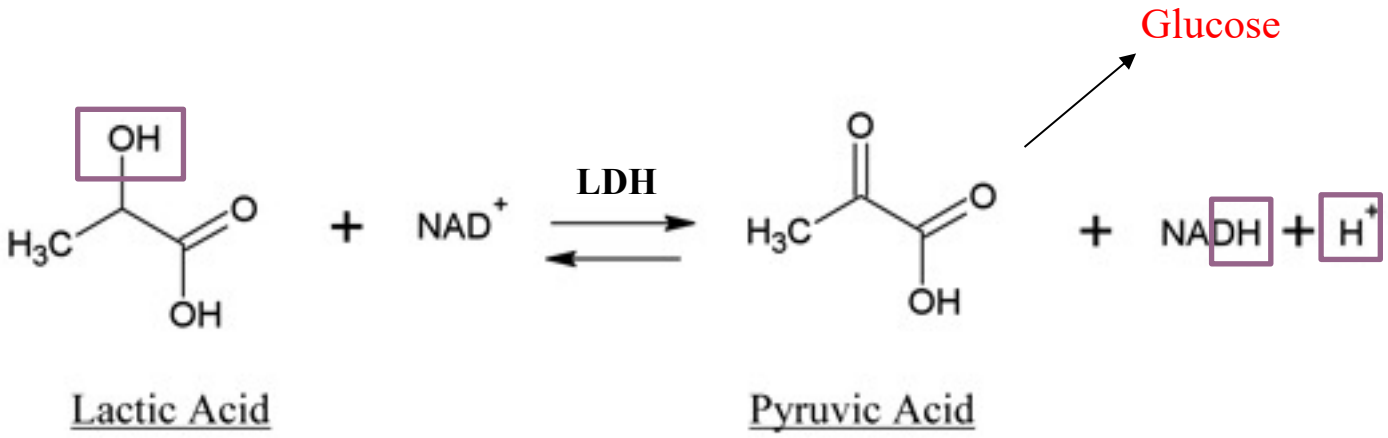
# Clinical Significance of Non-Functional Plasma Enzymes

**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)

- 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

# Lactate Dehydrogenase (LDH)

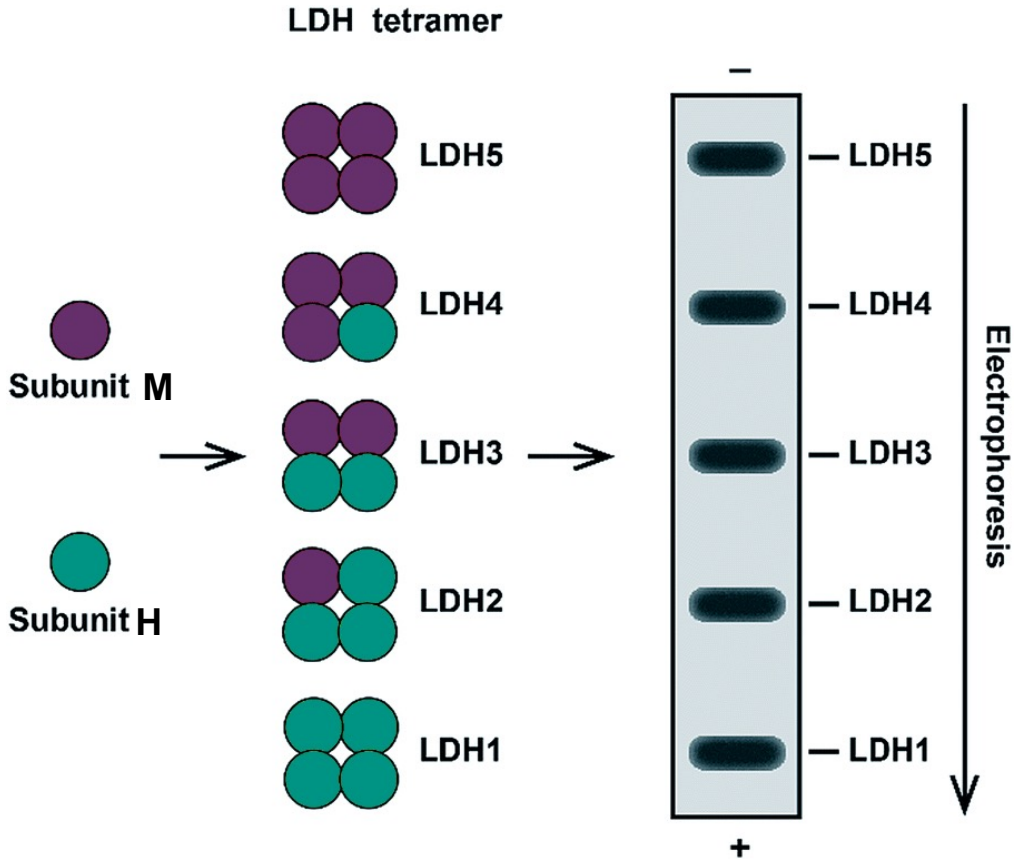
- **Lactic acid dehydrogenase (LDH)** is a cytoplasmic enzyme that helps produce energy.
- LDH is most often measured to evaluate the presence of tissue damage (diagnostic).
- The enzyme LDH is found in many body tissues, especially the **heart, liver, kidney, skeletal muscle, brain, blood cells and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

| High LDH Level in the Plasma in Different Diseases | 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 |

# LDH Isoenzymes

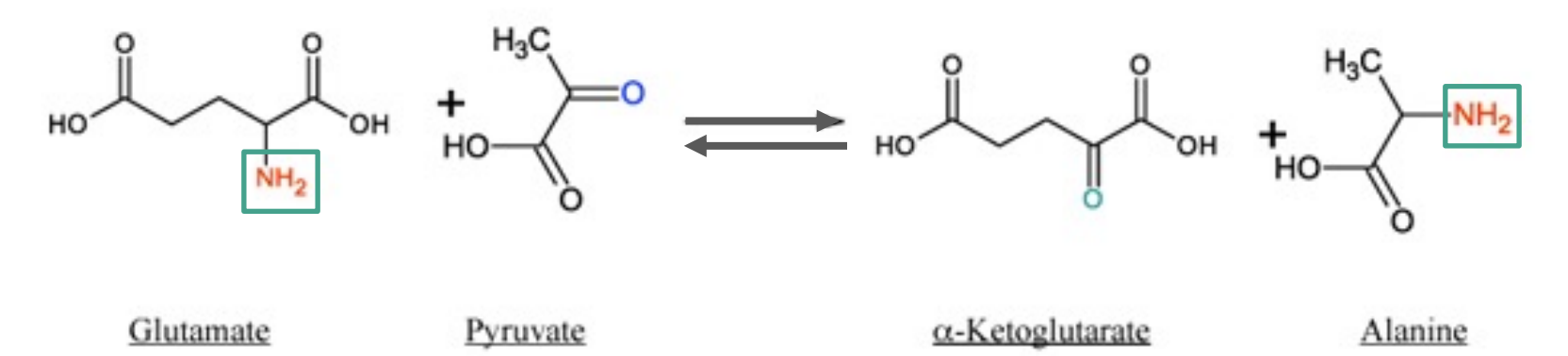
- LDH exists in **5 forms** (isoenzymes), which differ slightly in structure.
- All of these isoenzymes can be measured in the blood, and can be separated by electrophoresis.

| Isoenzyme | Cellular level | Diseases associated |
|---|---|---|
| LDH-1 | **Heart tissue** | Myocardial infarction |
| LDH-2 | **WBC** (monocytes), **RBC** | ➢ Megaloblastic anemia<br>➢ leukemia |
| LDH-3 | **Lung tissue** | Pulmonary embolism |
| LDH-4 | **kidneys**, placenta and **pancreas** | Pancreatitis |
| LDH-5 | **liver** and **skeletal muscle** | ➢ Toxic hepatitis with jaundice<br>➢ Muscular dystrophy |



# 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.
- Also called alanine transferase (**ALT**), serum glutamate-pyruvate transaminase (**SGPT**).




Glutamate Pyruvate α-Ketoglutarate Alanine

- **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

- Normally, high concentrations of ALT occur in the liver, and relatively low concentrations are found in the heart, muscle, and kidney.
- In the **serum**, only **low level** (10–35 U/L) of the ALT is found, thus an elevated level 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 💉

# Objectives

- To determine the level of **Lactate Dehydrogenase (LDH)** in serum
- To determine the level of **Alanine Transaminase (ALT)** in serum.
- To evaluate the presence of tissue damage.

# Lactate Dehydrogenase Assay

## Principle

**LDH** catalysis the following reaction:

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

- The rate of NADH formation is indicated by **increase the absorbance at 340 nm** and it is directly proportional to serum LDH activity.

If:

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

# Method

| | Pipette into cuvettes at 30 °C: |
|---|---|
| **Serum Sample** | 20 µl |
| **Buffer**<br>*Containing (TRIS buffer, Pyruvate) | 1000 µl |
| Mix and incubate at 30 °C for 3 minutes | |
| **Substrate**<br>*Containing (NADH) | 250 µl |
| Mix and incubate at 30 °C for 1 minute, then read the absorbance at 340 nm against distilled water (blank) every minute for 2 minutes. Then determine ΔA/min. | |

Measure enzyme kinetics using UV-visible spectroscopy:

**2) Applications → 2) Simple Kinetics → wavelength (340 nm) → 1) Seconds →Duration (120 sec) →Intervals (60 sec)→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 | |

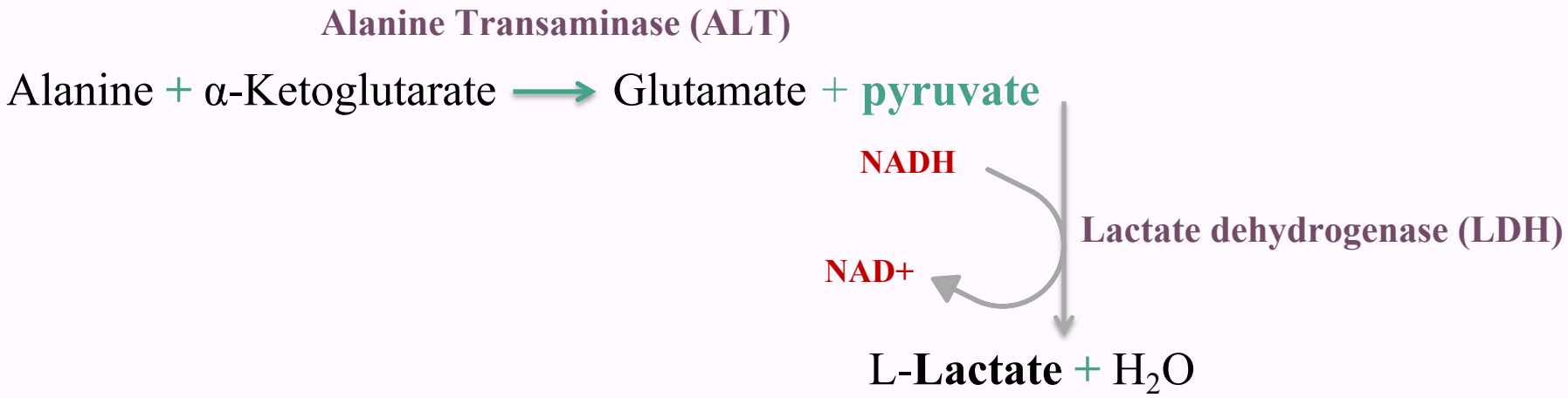
## Calculations

- **$\Delta A_1$, = $A_1 - A_2$** **$\Delta A_2 = A_2 - A_3$**
- **$\Delta$A/min** = ( $\Delta A_1 + \Delta A_2$) / 2
- **LDH Activity (U/L)** = $\Delta$A/min x 10080

**Normal Values:** Adults: 160 – 320 (U/L)

# Alanine Transaminase Assay

## Principle




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

**If:**

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

# Method

| | Pipette into cuvettes at 37 °C: |
|---|---|
| **ALT reagent**<br>*Containing (L-Alanine, Oxoglutarate LDH, NADH, buffer) | 1000 µl |

Pre-warm at 37 °C for 3 minutes and add

| | |
|---|---|
| **Sample (serum)** | 100 µl |

Mix and incubate at 37 °C for 1 minute, then read the absorbance at 340 nm against distilled water (blank) every minute for 2 minutes. Then determine ΔA/min.

Measure enzyme kinetics using UV-visible spectroscopy:

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## Calculations

- $\Delta A_1, = A_1 - A_2$ $\Delta A_2 = A_2 - A_3$
- **ΔA/min** = ( $\Delta A_1 + \Delta A_2$) / 2
- **ALT Activity (U/L)** = ΔA/min x 1768

**Normal Values:** Males: 10-40 (U/L). Female: 7- 35 (U/L).