

Determination of plasma enzymes

Determination of **LDH** in serum

Objectives

- To determine the level of those enzymes in serum as a tool for studying tissue function

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 levels help diagnose lung disease, lymphoma, anemia, and liver disease. They also help determine how well chemotherapy is working during treatment for lymphoma.

- The enzyme LDH is in many body tissues, especially

Heart

Liver

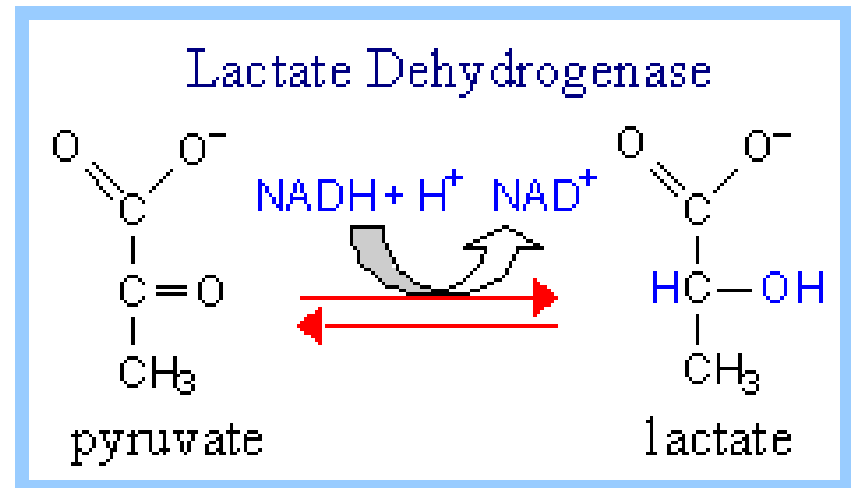
kidney

Skeletal muscle

brain

blood cells

lungs.



- Lactate is released into the blood and is eventually taken up by the liver.
- The liver converts lactate back to glucose and releases glucose into the blood.
- This glucose is then taken up by resting muscles, red blood cells, and other tissue.

- LDH exists in 5 forms (isoenzymes), which differ slightly in structure.

LDH Isoenzyme	Tissues or o
LDH-1	is found primarily in heart muscle and red blood cells.
LDH-2	is concentrated in white blood cells.
LDH-3	is highest in the lung.
LDH-4	is highest in the kidney, placenta, and pancreas
LDH-5	is highest in the liver
	and in skeletal muscle

All of these isoenzymes can be measured in the blood, and can be separated by electrophoresis.

– Principle

- LDH is a hydrogen transfer enzyme which catalyzes the interconversion of pyruvate and lactate.
- In the liver, it catalyzes the oxidation of L-lactate to pyruvate ($L \rightarrow P$) with the mediation of NAD as hydrogen acceptor.
- The reaction is reversible and the reaction equilibrium strongly favors the reverse reaction, namely the reduction of pyruvate to lactate ($P \rightarrow L$).
- The formation of NADH produces an increase in absorbance at 340 nm.
- The rate of absorbance change is directly proportional to the activity of LDH in the specimen.

2-Lactate Dehydrogenase Assay

Principle:

LDH catalysis the following reaction:



The rate of NADH formation is indicated by **increase** the **absorbance** at 340nm 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

	Tube
LDH reagent	3ml
Pre- warm at 37 c for 3 minutes	
Sample (serum)	0.1 ml(100 μ l)
Mix and incubate at 37 c for 1 min . Read the absorbance at 340 nm against distilled water every minute for 3 minute Determine $\Delta A/\text{min}$	

Calculation and Normal range

- LDH Activity= $\Delta A/\text{min} \times 4984 =$ U/L

NORMAL RANG OF LDH: (80-285) U/L male

(103- 277) U/L female

Thank You