

## Isoenzymes and their diagnostic significance

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<https://doi.org/10.5281/zenodo.10498361>

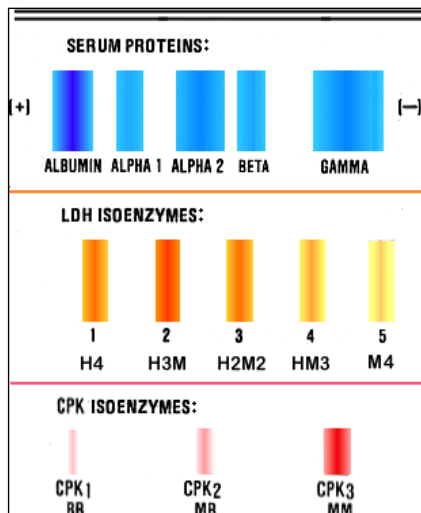
### Introduction

Multiple forms of same enzyme which differ in physical and chemical properties and catalyze the same reaction as an enzyme. Different isoenzymes may arise from different tissues and their specific detection may give clues to the site of pathology. Isoenzymes are produced by a single gene and some may result from more than one gene. Isoenzyme properties include:

- Catalyze the same reaction.
- Differ in AA sequence and physical properties.
- Separable on the basis of charge.
- Isoenzymes are tissue specific in their nature.

### Isoenzymes can be separated by:

- 1 Heat inactivation
- 2 Chemical inhibition
- 3 Electrophoresis technique (specific): Electrophoresis is a technique by which separation or movement of charged particle occurs when subjected to electrical field.



**Advantages of Isoenzyme measurement:**

1. Isoenzyme variants are derived from different tissue sources.
2. So, separation renders increased specificity to enzyme analysis.
3. Tissue or organ effects or changes can be detected (where isoenzyme elevation occurs).

1) **CPK (Creatinine Phospho Kinase)**

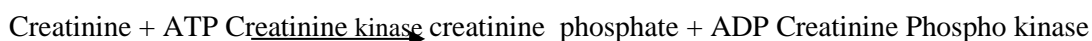
CPK Isoenzymes are performed when the total CPK level is elevated. Isoenzyme testing can help differentiate the source of the damaged tissue. CPK is an enzyme found predominantly in the heart, brain, and skeletal muscle. CPK is composed of 3 Isoenzymes that differ slightly in structure. CPK is a dimer made up of 2 subunits called- B for brain and M for muscle/skeletal muscle.

- CPK-1 (also called CPK-BB) is concentrated in the brain and lungs
- CPK-2 (also called CPK-MB) is found mostly in the heart
- CPK-3 (also called CPK-MM) is found mostly in skeletal muscle

Because the CPK-1 isoenzyme is predominately found in the brain and lungs, injury to either of these organs (for example, stroke or lung injury due to a pulmonary embolism) is associated with elevated levels of this isoenzyme.

Isoenzyme name	Composition	Present in	Elevated in
CK-1	BB	Brain	CNS disease
CK-2	MB	Myocardium, Heart	Acute myocardial infarction
CK-3	MM	Skeletal muscle, Myocardium	

Creatinine Phospho kinase or Creatinine Kinase catalyses the conversion of creatinine-to-creatinine Phosphate.



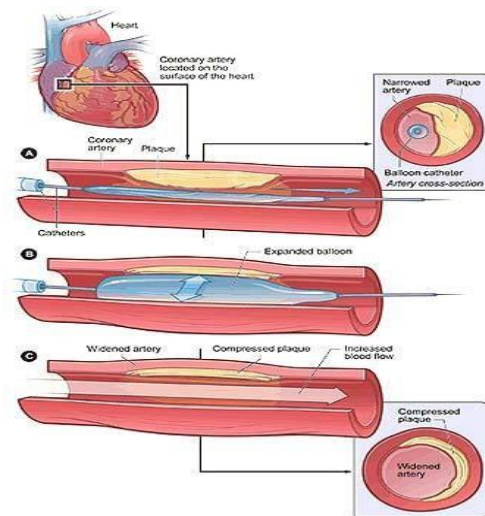
Normal level: 15-100U/L (males)  
: 10-80 U/L (females)

**Clinical Significance of CPK (CK)-**

- In heart attack, atherosclerosis
- CPK2 isoenzymes is very small (2% of total CPK activity) & undetectable in plasma



- In myocardial infarction, CPK2 levels are increased within 4 hrs, then falls rapidly
- Total CPK level is elevated upto 20 folds in myocardial infarction
- It is present in serum when there is extensive tissue damage causing breakdown of mitochondria and cell wall.
- Its presence in serum indicates cellular damage, seen in malignancies.
- **Atherosclerosis:** Is a condition in which arteries are blocked to a greater or lesser extent by deposition of cholesterol plaques, leading most commonly to coronary heart disease by blocking of coronary arteries i.e (myocardial infarction MI).



## 2) LDH (Lactate Dehydrogenase)



- LDH is elevated in myocardial infarction, blood disorders.
- It is a tetrameric protein and made of two types of subunits namely H = Heart, M = skeletal muscle
- It exists as 5 different isoenzymes with various combinations of H and M subunits.
- Normal values: Serum- 100-200 U/L  
CSF- 7-30 U/L  
Urine- 40-100 U/L
- The LDH has five Isoenzymes which are:
  - LDH-1 (H<sub>4</sub>) is found mainly in the heart.
  - LDH-2 (H<sub>3</sub>M<sub>1</sub>) Reticuloendothelial system.



LDH-3 (H2M2) is found in the lungs.

LDH-4 (H1M3) in the kidney, placenta, and pancreas

LDH-5 (M4) in liver and striated (skeletal) muscle.

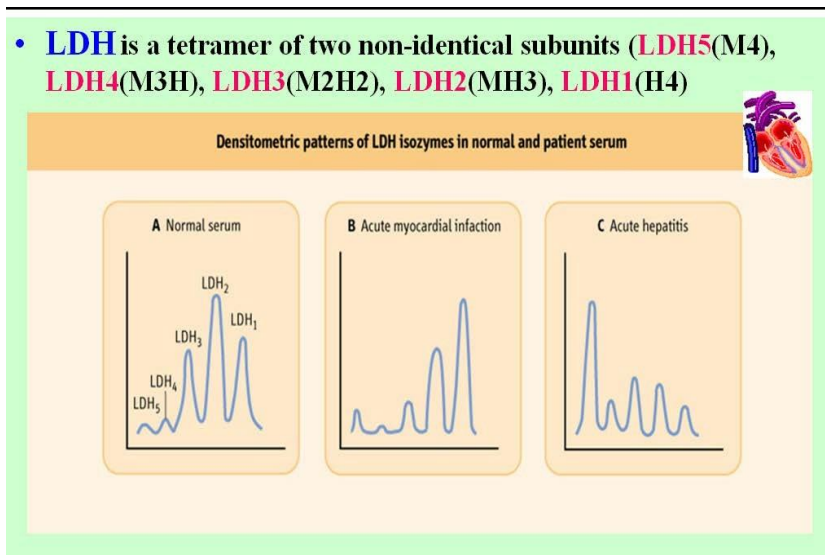
- Normally, levels of LDH-2 are higher than those of the other isoenzymes
- LDH is found in the cells of almost all body tissues.
- Because this enzyme is actually composed of five different isoenzymes, however, analysis of the different LDH isoenzyme levels in the blood can help in the diagnosis of some diseases.
- LDH is an oxidoreductase enzyme whose activity is necessary for the reversible reaction in which Pyruvate and lactate are interconverted. It is important in glycolysis.
- LDH isoenzyme is a tetramer with 4 subunits. The subunit may be either H (heart) or M (muscle).

Isoenzyme name	Composition	Present in	Elevated in
LDH1	(H4)	Myocardium, RBC	Myocardial infarction
LDH2	(H3M1)	Myocardium, RBC	
LDH3	(H2M2)	Skeletal muscle, Kidney	
LDH4	(H1M3)	Skeletal muscle, Kidney	
LDH5	(M4)	Skeletal muscle, Liver	Skeletal muscle and liver disease

#### **Clinical Significance of CPK (CK)-**

- In normal serum, LDH2 (H3M) predominant enzyme and LDH5 is rarely seen.
- In myocardial infarction, LDH1 (H4) levels are greater than LDH2.
- Megaloblastic anaemia (50 times upper limit of LDH1 and LDH2).
- Muscular dystrophy, LDH5 (M4) is increased.
- Toxic hepatitis with jaundice (10 times more LDH5).
- Renal disease- tubular necrosis or pyelonephritis, pulmonary embolism LDH3 (massive destruction of platelets)
- Total LDH is increased in neoplastic diseases.
- LDH5 increased in breast cancer, malignancies of CNS, prostatic carcinoma.
- In leukaemias, LDH2 and LDH3 levels are increased.
- In malignant tumors of testis and ovaries, LDH2, LDH3 and LDH5 levels are increased.





### LDH and heart attack

#### 3) ALP (Alkaline phosphatase)

- Isoenzymes are five:
    1. ALP-1 present in liver increased in obstructive jaundice, biliary cirrhosis.
    2. ALP-2 in bone increased in rickets and osteomalacia
    3. ALP-3 in placenta increase in 2<sup>nd</sup> and 3<sup>rd</sup> trimester of pregnancy and decrease indicates placental insufficiency and foetal death.
    4. ALP-4 in intestine increased in intestinal disease and after gastrectomy surgery
    5. ALP-5 in kidney increases in kidney disorders.
- Normal level: 40-125 U/L

#### 4) Amylase

- Two Isoenzymes:
  1. Salivary in normal serum 60% increase in Parotitis/ mumps
  2. Pancreatic 40% increase in pancreatitis
- The peak levels are seen between 5-12hours after onset of disease and returns to normal level within 2-4 days.
- Normal level: 50-120 IU/L

#### 5) Aldolase

- Three Isoenzymes:
  1. Aldolase-A present in muscle increased in muscular dystrophies.
  2. Aldolase-B in liver increased in hepatitis.
  3. Aldolase-C in brain
- Normal level: 1.5-7 U/L



6) **Acid phosphatase**

- Present in Prostate, RBC and platelets.
- Increased in Prostate cancer, Gaucher's disease (fatty substances in liver and spleen) and thromboembolic disorders
- Normal level: 2.5-12 U/L

**Enzymes in liver diseases:**

- The following enzymes when elevated are useful in the diagnosis of liver diseases and disfunction due to viral hepatitis, toxic hepatitis, cirrhosis and hepatic necrosis
1. Alanine transaminas(ALT).
  2. Aspartate transaminase (AST).
  3. Lactate dehydrogenase (LDH).
- The enzymes that markedly increase in intrahepatic and extra hepatic cholestasis are
- 1) Alkaline Phsphatase and
  - 2) Nucleotidase.

