Enzymes are organic molecules that accelerate biochemical reactions but emerge from the reaction unchanged. Abnormal levels of plasma enzymes are highly suggestive of damaged cells and provide clues to parts of the body that may be involved in disease processes. Enzyme levels are measured in the clinical laboratory to identify the site of damage and to quantify the amount of damage. This paper will discuss the physiological sources, clinical significance, and laboratory-testing methodology for lactate dehydrogenase (LD), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT).

**Keywords:** enzymes, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyltransferase

The aminotransferases are a group of enzymes that catalyze the conversion of amino acids to 2-oxo-acids by transfer of amino groups. The highest levels of aspartate aminotransferase (AST) are present in liver, cardiac tissue, and skeletal muscle, with smaller amounts present in the kidneys, pancreas, and erythrocytes. Alanine aminotransferase (ALT), however, is predominantly present in the liver.

Alkaline phosphatase (ALP) catalyzes the hydrolysis of various phosphomonoesters at an alkaline pH. The highest concentrations of ALP are found in bone, liver, spleen, intestine, placenta, and kidneys. ALP contains a number of isoenzymes, with bone, liver, and placenta types being the most extensively studied. Isoenzyme analysis is of limited clinical use due to ineffective methods of separation and the availability of more efficient tests for clinical diagnosis.

Gamma-glutamyltransferase (GGT) catalyzes the transfer of the γ-glutamyl residue from γ-glutamyl peptides to amino acids; this epithelial enzyme is responsible for the catabolism of extracellular glutathione. GGT is present in kidney, brain, prostate, pancreas, and liver tissue.

**Clinical Significance**

LD levels are increased in several disorders due to its presence in a variety of tissues; the most dramatic
increase is observed in prehepatic disorders, hemolytic disease, testicular and germ cell tumors, and acute myocardial infarction (MI). LD levels increase 12 hours after an MI and peak in 2 days, returning back to normal levels in 7 to 14 days. Normally, plasma LD2 levels predominate in blood, followed by LD1 levels; however, damaged cardiac tissue in MI causes LD1 levels to increase, which triggers a reversal in which LD1 levels become greater than LD2 levels. Isoenzyme analysis has now become outdated due to the availability of more sensitive and specific cardiac markers such as troponin for the diagnosis of MIs. Currently, LD is mostly used by laboratorians as a nonspecific screening or monitoring tool to determine the extent of tissue damage. The Reference range for LD is 125 to 220 U/L.2

ALT and AST are clinically significant aminotransferases.3 Both are markers of liver disease; however, ALT is more liver specific than AST.4 AST levels are also significantly increased in skeletal muscular disorders and pulmonary embolism. In acute inflammatory conditions of the liver, ALT levels are higher than AST levels because ALT has a half-life of 16 to 24 hours.2 Moreover; increased ALT levels have been associated with deaths from liver disease and all-cause mortality in some studies. The reference range for ALT is 7 to 45 U/L and that of AST is 5 to 35 U/L.2

ALP levels are highly increased in hepatobiliary and bone disorders. Levels are normally increased during pregnancy and in growing children due to the placental and bone fractions, respectively. Intestinal ALP levels increase in disorders of the digestive tract and liver. ALP levels are decreased in inherited hyperphosphatasia.2 The reference range for ALP varies with age and sex, with higher levels for younger compared with older individuals and for men compared to women, probably due to bone-fraction measurements.

GGT levels are mainly evaluated in hepatobiliary and liver disorders. Levels are also increased in patients with chronic alcoholism who take enzyme-inducing drugs such as warfarin and phenytoin, as well as in conditions such as pancreatitis and diabetes mellitus.2,7 Moreover, high GGT levels are also associated with high rates of all-cause mortality.5 The reference range for GGT is 6 to 55 U/L for men and 5 to 38 U/L for women. Values are lower in females due to higher levels of estrogen and progesterone.2

Testing Methods and Interferences

The measurement of enzymes is standardized, simple, and inexpensive; it requires no special preparation for the patient.1 Due to the small quantity of enzymes normally available in blood, the rate of reaction is measured photometrically, related to the activity of the enzyme. This enzyme activity is proportional to the concentration of the enzyme.2 Serum or heparinized plasma is the preferred type of sample for enzyme analysis.

Laboratorians can measure LD activity by gauging the absorbance of coenzyme nicotinamide adenine dinucleotide (NAD) as it is changed to reduced nicotinamide adenine dinucleotide (NADH) while lactate is oxidized to pyruvate in the following reaction:

\[ \text{Lactate} + \text{NAD}^{+} \rightleftharpoons \text{Pyruvate} + \text{NADH} + \text{H}^{+} \]

This reaction is derived from glycolysis, which is the biochemical pathway for metabolism of glucose in all living cells. Samples must be free of hemolysis because LD levels are 100- to 150-fold higher in erythrocytes than in serum. Also, samples must be stored at room temperature and analyzed within 48 hours of sample collection.2

AST and ALT activity are measured by measuring the change in absorbance as aspartate and alanine transfer their amino group to the appropriate respective α-keto acids. Pyridoxal-5-phosphate is the coenzyme for these reactions.

\[ \text{Aspartate} + \alpha - \text{Ketoglutarate} \rightleftharpoons \text{Oxaloacetate} + \text{Glutamate} \]
\[ \text{Oxaloacetate} + \text{NADH} + \text{H}^{+} \rightleftharpoons \text{Malate} + \text{NAD}^{+} \]
\[ \text{Alanine} + \alpha - \text{Ketoglutarate} \rightleftharpoons \text{Pyruvate} + \text{Glutamate} \]
\[ \text{Pyruvate} + \text{NADH} + \text{H}^{+} \rightleftharpoons \text{Lactate} + \text{NAD}^{+} \]

Change in absorbance is directly proportional to the concentration of ALT and AST.2

ALT and AST reactions are extremely important for synthesis and degradation of amino acids; oxaloacetate and pyruvate are oxidized by the tricarboxylic cycle (Krebs cycle) to provide intermediate products that generate energy for living cells. Samples must be free of hemolysis for AST; however, ALT levels are unaffected by any interference from erythrocytes. For best results, samples
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must be stored at refrigerated temperatures after collection and be analyzed within 3 to 4 days.²

ALP activity is measured by a method devised by Bowers and McComb⁸ that involves calculation of activity based on molar absorptivity of \( p \)-nitrophenol. Para-nitrophenylphosphate, a colorless compound, is hydrolyzed to a yellow-colored \( p \)-nitrophenol, and increase in absorbance can be measured in the following reaction:

\[
p - Nitrophenylphosphate \leftrightarrow p - Nitrophenol + Phosphatean

The increase in absorbance of \( p \)-nitrophenol is directly proportional to ALP activity. Samples collected for ALP analysis must be free of hemolysis and must be analyzed soon after collection. Ingestion by the patient of a high-fat meal before specimen collection may also cause falsely increased values due to the intestinal fraction.² However, fasting specimens are not recommended because the interference is negligible.

GGT activity is measured when \( \gamma \)-glutamyl-\( p \)-nitroanilide transfers its \( \gamma \)-glutamyl residue to glycylglycine. This results in the formation of a chromagen, \( p \)-Nitroaniline, as shown in the following reaction:

\[
\gamma - Glutamyl - p - nitroanilide + Glycylglycine 
\leftrightarrow \gamma - Glutamyl - glycylglycine + p - Nitroaniline
\]

The absorbance of \( p \)-Nitroaniline is directly proportional to the activity of GGT, which is stable in serum samples for 1 week at refrigerated temperatures.

Overall, LD, ALT, AST, ALP, and GGT are enzymes that are clinically significant in various disorders including conditions of the liver. LD levels are most predominant in pre-hepatic and other hemolytic disorders due to its presence in erythrocytes. ALT and AST are predominant in hepatic disorders, with ALT being more liver specific than ALT. ALP and GGT are increased in posthepatic disorders with GGT more so with chronic alcoholism. Increased activity of these enzymes provides clues to the source of the clinical problem and can be extremely valuable in diagnosis of the patient’s condition. LM

References