ALKALINE PHOSPHATASE,
ACID PHOSPHATASE,
ALANINE AMIOTRANSFERASE,
ASPARTATE AMINOTRANSFERASE,
LACTATE DEHYDROGENASE,
CREATINE PHOSPHOKINASE.

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Alkaline phosphatase (ALP) is an enzyme found in several tissues throughout the body. The highest concentrations are present in the cells bone and the liver. Normal range 45 - 150 U/L, This test measures the level of ALP in the serum.

- In the liver, ALP is found on the edges of cells that join to form bile ducts, tiny tubes that drain bile from the liver to the bowels, where it is needed to help digest fat in the diet.
- ALP in bone is produced by special cells called osteoblasts that are involved in the formation of bone. Each of the various tissue types produces distinct forms of ALP called isoenzymes.

ALP blood levels can be greatly increased, in cases where

- one or more bile ducts are blocked. This can occur as a result of inflammation of the gallbladder (cholecystitis) or gallstones. Smaller increases of blood ALP are seen in liver cancer and cirrhosis, with use of drugs toxic to the liver,

and in hepatitis. when a person has signs and symptoms of liver involvement may include: Weakness, fatigue , Loss of appetite, Nausea, vomiting, Abdominal swelling and/or pain, Jaundice, Dark urine, light-colored stool, Itching (pruritus).

- Any condition causing excessive bone formation, including bone disorders such as Paget's disease, can cause increased ALP levels. Or other bone conditions, such as vitamin D deficiency.

Some examples of the signs and symptoms suggesting a bone disorder include:

Bone and/or joint pain, Increased frequency of fractures, Deformed bones

- Children and adolescents typically have higher blood ALP levels because their bones are still growing.
- Some drugs may affect ALP levels. For example, oral contraceptives may decrease levels while anti-epileptics may increase levels.

Alkaline phosphatase assay The kit uses p-nitrophenyl phosphate (pNPP) as a phosphatase substrate which turns yellow ($\lambda max = 405$ nm) when dephosphorylated by ALP

Alkaline phosphatase assay protocol summary:

- add samples and standards to wells
  - add pNPP solution to sample wells (not to standards)
  - add ALP enzyme solution to standard wells (not to samples)
  - incubate for 60 min at room temp
  - add stop solution
  - analyze with microplate reader.

Acid phosphatase (AP): It can be further classified as a phosphomonoesterase. Acid phosphatase is stored in lysosomes and functions when these fuse with endosomes, which are acidified while they function; therefore, it has an acid pH optimum. An enzyme that acts to liberate phosphate under acidic conditions and Different forms of acid phosphatase are found in different organs in the liver, spleen, bone marrow, and prostate gland. Normal range 0--0.8 U/L Abnormally high serum levels of acid phosphatase may indicate infection, injury, or cancer of the prostate. and their serum levels are used to evaluate : in

- It's also used as a cytogenetic marker to distinguish the

different lineages of Acute Lymphoblastic Leukemia(ALL)

- acid phosphatase may be used as a biochemical marker of osteoclast function during the process of bone resorption
- The discovery that prostatic carcinoma cells often retain a high concentration of acid phosphatase characteristic of the normal postpubertal gland led to the recognition of the first clinically useful tumor marker.

**Aspartate aminotransferase (AST):** is an enzyme found in cells throughout the body but mostly in the heart and liver and, to a lesser extent, in the kidneys and muscles. When liver or muscle cells are injured, they release AST into the blood. the range for normal AST is reported between 10-40 units per liter

A number of conditions can cause injury to liver cells and may cause increases in AST. The test is most useful in detecting liver damage due to hepatitis, drugs toxic to the liver, cirrhosis, or alcoholism. AST, however, is not specific for the liver and may be increased in heart, kidneys and muscles injury. An AST test is often performed along with an alanine aminotransferase (ALT) test

although ALT is more specific for the liver than is AST. Both are enzymes found in the liver that become elevated in the blood when the liver is damaged.

A calculated AST/ALT ratio is useful for differentiating between different causes of liver injury and in recognizing when the increased levels may be coming from another source, such as heart or muscle injury. In most types of liver disease, the ALT level is higher than AST and the AST/ALT ratio will be low (less than 1). There are a few exceptions; the AST/ALT ratio is usually increased in alcoholic hepatitis, cirrhosis, hepatitis C virus-related chronic liver disease, and in the first day or two of acute hepatitis or injury from bile duct obstruction.

With heart or muscle injury, AST is often much higher than ALT (often 3-5 times as high) and levels tend to stay higher than ALT for longer than with liver injury.

AST levels are often compared with results of other tests such as alkaline phosphatase (ALP), total protein, and bilirubin to help determine which form of liver disease is present.

**Sometimes AST may be used to monitor people who are taking medications that are potentially toxic to the liver.
**when a person has signs and symptoms of a liver disorder. Some of these may include: Weakness, fatigue Loss of appetite, Nausea, vomiting Abdominal swelling and/or pain, Jaundice Dark urine, light-colored stool, Itching (pruritus) Swelling in the legs and ankles,
**people who are at an increased risk for liver disease since many people with mild liver damage will have no signs or symptoms. Some examples include:

- Persons who might have been exposed to hepatitis viruses
- Persons who are heavy drinkers
- Persons who have a history of liver disease in their family
- Persons taking drugs that can damage the liver
- Persons who are overweight and/or have diabetes
- Pregnancy,
- a shot or injection of medicine into muscle tissue, or even strenuous exercise may increase AST levels. Acute burns, surgery, and seizures may raise AST levels as well.

Aspartate Aminotransferase (AST) assay protocol summary: It catalyzes the reaction: Aspartate + α-Ketoglutarate ⇌ Oxaloacetate + Glutamate Diagnostically, it is almost always measured in units/liter (U/L). AST Assay Kit, an amino group is transferred of from aspartate to α-ketoglutarate. The products of this reversible transamination reaction are oxaloacetate and glutamate. The glutamate is detected in a reaction that concomitantly converts a nearly colorless probe to color (λmax = 450 nm). The kit provides a rapid, simple, sensitive and reliable test suitable as a high throughput activity assay of AST with a detection limit of 10 mU per well.

Alanine aminotransferase (ALT) is an enzyme found mostly in the cells of the liver and kidney. Much smaller amounts of it are also found in the heart and muscles. This test measures the level of ALT in the serum. When the liver is damaged, ALT is released into the blood, usually before more obvious signs of liver damage occur, such as jaundice or hepatitis or as a result of drugs or other substances that are toxic to the liver. The reference range for ALT is 20-60 IU/L

Very high levels of ALT (more than 10 times normal) are usually due to acute hepatitis, sometimes due to a viral infection. Levels of ALT may also be markedly elevated (sometimes over 100 times normal) as a result of exposure to drugs or other substances that are toxic to the liver or in conditions that cause decreased blood flow (ischemia) to the liver.

ALT levels are usually not as high in chronic hepatitis, often less than 4 times normal. Other causes of moderate increases in ALT include obstruction of bile ducts, cirrhosis (usually the result of chronic hepatitis or bile duct obstruction), heart damage, alcohol abuse, and with tumors in the liver.

Alanine aminotransferase assay protocol summary:

ALT Assay Kit, ALT catalyzes the transfer of an amino group from alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate. The pyruvate is detected in a reaction that concomitantly converts a nearly.

colorless probe to both color (λmax = 570 nm) and fluorescence (Ex/Em = 535/587 nm). The kit provides a rapid, simple, sensitive, and reliable test suitable for high throughput activity assay of ALT with a detection limit of 10 mU per well

**A lactate dehydrogenase (LD or LDH) :** test is a non-specific test that may be used in the evaluation of a number of diseases and conditions Older than 12 years 300 - 600 U/L

An elevated level of LD may be seen with:

- To detect and monitor progressive conditions muscle trauma or injury, or severe infections.
- To help stage, determine prognosis, and/or monitor treatment (i.e., chemotherapy) of cancers.
- This test may be ordered, for example, when a person has signs and symptoms of meningitis or when someone has a buildup of fluid around the heart, lungs or in the abdomen.

❑ LD levels typically will rise as the cellular destruction begins, peak after some time period, and then begin to fall.

- Strenuous exercise can cause temporary elevations in LD.
- Hemolysis of the blood specimen can cause falsely elevated results.
- If a person's platelet count is increased, serum LD can be artificially high and not reflective of the LD actually present in the circulation.

A lactate dehydrogenase assay protocol summary

First, LDH catalyzes the conversion of lactate to pyruvate via reduction of NAD+ to NADH. Second, diaphorase uses NADH toreduce a tetrazolium salt (INT) to a red formazan product. Therefore, the level of formazan formation is directly proportional to the amount of released LDH in the medium.

The assay is performed by transferring cell culture media from treated cells into a new microplate and adding the kit reagents. After incubation at room temperature for 30 minutes, reactions are stopped and LDH activity is determined by spectrophotometric absorbance at 490nm.

A creatine kinase (CK): normal range is 22 to 198 U/L, test may be used to detect

- inflammation of muscles (myositis) or muscle damage due to muscle disorders (myopathies) such as muscular dystrophy or to help diagnose rhabdomyolysis.
- CK may be ordered along with other blood chemistry tests such as electrolytes, BUN or creatinine (to evaluate kidney function). Dark urine (The urine may be dark because of the presence of myoglobin, another substance released by damaged muscles that can be harmful to the kidneys).
- may order CK have muscle injury with few or nonspecific symptoms, such as weakness, fever, and nausea.

- May use a CK test especially if someone is taking a drug such as a statin, using ethanol or cocaine, or has been exposed to a known toxin that has been linked with potential muscle damage.
- The test may be ordered when a person has symptoms associated with Increased CK may be seen with, for example:

Recent crush and compression muscle injuries, trauma, burns, and electrocution, Inherited myopathies, such as muscular dystrophy, Hormonal (endocrine) disorders, such as thyroid disorders, Addison disease or Cushing disease, Strenuous exercise, Prolonged surgeries, Seizures, Infections – viral (such as influenza and HIV), bacterial, fungal, and parasitic (such as malaria ,Connective tissue disorders (e.g. lupus, rheumatoid arthritis), Celiac disease, Renal failure

The three types of CK isoenzymes : CK-MB (found primarily in heart muscle), CK-MM (found primarily in skeletal muscle), and CK-BB(found primarily in the brain; when present in the blood, it is primarily from smooth muscles, including those in intestines, uterus or placenta).

- People who have greater muscle mass have higher CK, men generally tend to have higher values than women.

## the creatine kinase assay protocol,:

creatine kinase (CK) converts creatine into phosphocreatine and ADP. The phosphocreatine and ADP then react with the CK enzyme mix to form an intermediate, which reduces a colorless probe to a colored product with strong absorbance at λ= 450 nm.

Creatine kinase assay protocol summary:

- add samples and standards to wells
- add reaction mix
- analyze every 1-2 min for 10-40 min with microplate reader in kinetic mode at 37°C