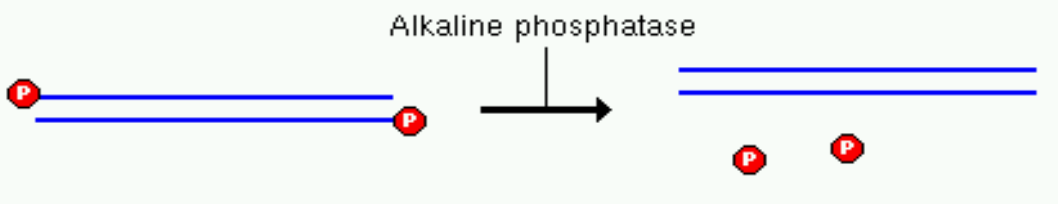


ALKALINE PHOSPHATASE

Alkaline Phosphatases are a group of enzymes found primarily the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). What is measured in the blood is the total amount of alkaline phosphatases released from these tissues into the blood. As the name implies, this enzyme works best at an alkaline pH (a pH of 10), and thus the enzyme itself is inactive in the blood. Alkaline phosphatases act by splitting off phosphorus (an acidic mineral) creating an alkaline pH.

Alkaline phosphatase removes 5' phosphate groups from DNA and RNA. It will also remove phosphates from nucleotides and proteins. These enzymes are most active at alkaline pH - hence the name.



There are several sources of alkaline phosphatase that differ in how easily they can be inactivated:

- Bacterial alkaline phosphatase (BAP) is the most active of the enzymes, but also the most difficult to destroy at the end of the dephosphorylation reaction.
- Calf intestinal alkaline phosphatase (CIP) is purified from bovine intestine. This is phosphatase most widely used in molecular biology labs because, although less active than BAP, it can be effectively destroyed by protease digestion or heat (75C for 10 minutes in the presence of 5 mM EDTA).
- Shrimp alkaline phosphatase is derived from a cold-water shrimp and is promoted for being readily destroyed by heat (65C for 15 minutes).

There are two primary uses for alkaline phosphatase in DNA manipulations:

- Removing 5' phosphates from plasmid and bacteriophage vectors that have been cut with a restriction enzyme. In subsequent ligation reactions, this treatment prevents self-ligation of the vector and thereby greatly facilitates ligation of other DNA fragments into the vector (e.g. subcloning).
- Removing 5' phosphates from fragments of DNA prior to labeling with radioactive phosphate. Polynucleotide kinase is much more effective in phosphorylating DNA if the 5' phosphate has previously been removed.

It is usually recommended that dephosphorylation of DNAs with blunt or 5'-recessed ends be conducted using a higher concentration alkaline phosphatase or at higher temperatures than for DNAs with 5' overhangs

The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. Since the mucosal cells that line the bile system of the liver are the source of alkaline phosphatase, the free flow of bile through the liver and down into the biliary tract and gallbladder are responsible for maintaining the proper level of this enzyme in the blood. When the liver, bile ducts or gallbladder system are not functioning properly or are blocked, this enzyme is not excreted through the bile and alkaline phosphatase is released into the blood stream. Thus the serum alkaline phosphatase is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine.

In addition to liver, bile duct, or gallbladder dysfunction, an elevated serum alkaline phosphatase can be due to rapid growth of bone since it is produced by bone-forming cells called osteoblasts. One would expect that growing children have higher levels than full-grown adults. The relationship of alkalinity to bone development warrants further discussion because it plays a major role in the prevention and reversal of osteoporosis. Just as calcium builds up around faucets, so is calcium laid down into bone. The reason the calcium deposits on your faucet is because the water is alkaline and calcium comes out of

solution and crystallizes in an alkaline environment. The reverse is also true, "Lime -Away", vinegar, or any other acidic solution dissolve the calcium deposits because they are acidic. It makes sense that osteoblasts by creating a local environment of alkalinity via alkaline phosphatase helps build bone. It also implies that in order to slow bone loss, one can not be in an acidic state. Studies have shown that giving bicarbonate of potassium is just as effective as calcium in correcting osteoporosis! One would expect then that in an acidic state, the body will compensate for this by increasing the bone alkaline phosphatase levels.

Because acid-alkaline is influenced by many other glands, the implications of serum alkaline phosphatase levels must consider more than just bone and liver function. Associated organs/glands include adrenals, uterus, prostate, and intestine.

The consequences of impaired bile flow are pervasive since bile is critical to your body's ability to process fats. As a result, fats remain undigested in the digestive tract and can cause bloating, cramps, light colored stools, gaseousness, etc. especially after a rich food. Many patients report pressure or pain in the right upper area of their abdomen where the liver and gallbladder are located. You may have discomfort in the right shoulder or between your shoulder blades anywhere from your mid-back to the base of your neck. Many people say they "carry my stress in the upper back and neck." This may due to gallbladder dysfunction. Unfortunately, a normal alkaline phosphatase does not exclude hepatobiliary dysfunction. In many cases, even the ultrasound shows no gallstones, etc. Rather the problem is that the bile does not flow freely throughout the system, which may result in insufficient bile action.

The consequences of impaired bile function involve the endocrine system in a major way because all of the steroid hormones are metabolized in part by the liver. These include the sex hormones (androgens and estrogens). As a result the menstrual cycle, sexual functions and sex characteristics can be affected.

The optimal range for alkaline phosphatase depends on your age. A growing adolescent will have a much higher alkaline phosphatase than a full grown adult because his/her osteoblasts are laying down bone very rapidly. For an adult, 50-75 mg/dl is considered a reasonable optimal range.

An **increased** serum Alkaline Phosphatase may be due to: Congestion or obstruction of the biliary tract, which may occur within the liver, the ducts leading from the liver to the gallbladder, or the duct leading from the gallbladder through the pancreas that empty into the duodenum (small intestine). Any of these organs (liver, gallbladder, pancreas, or duodenum) may be involved.

Liver congestion/cholestasis

- Oral contraceptives
- Obstructive pancreatitis
- Hepatitis/Mononucleosis/CMV
- Congestive heart failure
- Parasites
- Malignancy involving liver

Osteoblastic/Bone Conditions

- Herpes Zoster (Shingles)
- Hyperthyroidism
- Over-activity of the Parathyroid glands (Primary Hyperparathyroidism, Secondary Hyperparathyroidism from kidney disease, osteomalacia, malabsorption)
- Rickets - Vitamin D deficiency
- Healing fractures, rapid bone growth such as after a fracture, bone cancers like osteogenic sarcoma, Osteomalacia, and Paget's disease.
- Osteoporosis treatment
- Adrenal cortical hyperfunction

Non-Bone/Non-Liver Conditions

- As a normal part of late pregnancy since the placenta produces alkaline phosphatase (placenta - ~2x normal)
- Amyloidosis
- Granulation tissue
- Gastrointestinal inflammation (Inflammatory Bowel Disease: Ulcerative colitis, Crohn's; ulcers)
- Systemic infections (sepsis)
- Sarcoidosis.
- Rheumatoid arthritis.
- Certain cancers such as Hodgkin's Lymphoma, gynecologic malignancies.
- Acute tissue damage in the heart or lungs (myocardial or pulmonary infarctions).

An elevated alkaline phosphatase almost always requires other tests to determine the origin of the condition. For example, liver enzyme tests to check the integrity of the liver, x-rays or other bone images if a bone abnormality is evident. Although not used often, the isoenzyme profile of alkaline phosphatases can be determined to see if the elevation of alkaline phosphatase came primarily from liver (ALP-1), bone (ALP-2), or elsewhere. Most often, however there is a modest elevation from ideal but the actual value is within the laboratories reference range and the origin is inferred from the symptoms, exam, or existing lab results.

A **decreased** serum alkaline phosphatase may be due to:

- Zinc deficiency.
- Hypothyroidism.
- Vitamin C deficiency/Scurvy.
- Folic acid deficiency.
- Excess Vitamin D intake.
- Low phosphorus levels (hypophosphatasia)
- Celiac disease.
- Malnutrition with low protein assimilation (including low stomach acid production/hypochlorhydria).
- Insufficient Parathyroid gland function.

- Pernicious anemia
- Vitamin B₆ insufficiency

Alkaline phosphatase is an enzyme which catalyzes the hydrolysis of phosphate monoesters. It is membrane-bound and widely found:

- liver - 55%; in hepatocytes next to biliary canaliculi
- bone - 45%; in osteoblasts
- gut - 5%
- kidney
- placenta

The liver, bone and kidney enzymes are different forms of the same gene product; the gut and placental forms are isoenzymes.

Total serum alkaline phosphatase is normally measured but liver and bone isoenzymes may be distinguished if required. They are normally present in roughly equal amounts within plasma. Gut ALP rises after meals, especially in those of blood group B or O who are secretors of blood group substances. Bone ALP increases at the time of physiological growth spurts. Placental ALP normally increases at the end of the third trimester.

Bacterial

In bacteria, alkaline phosphatase is located in the periplasmic space, external to the cell membrane. Since this space is much more subject to environmental variation than the actual interior of the cell, bacterial alkaline phosphatase is comparatively resistant to inactivation, denaturation, and degradation, and also has a higher rate of activity. Although the actual purpose of the enzyme is still not fully understood, the simple hypothesis, that it is a means for the bacteria to generate free phosphate groups for uptake and use, is supported by the fact that alkaline phosphatase is usually only produced by the bacteria during phosphate starvation and not when phosphate is plentiful. However, other possibilities exist; for instance,

the presence of phosphate groups usually prevents organic molecules from passing through the membrane, therefore dephosphorylating them may be important for bacterial uptake of organic compounds in the wild. Some complexities of bacterial regulation and metabolism suggest that other, more subtle, purposes for the enzyme may also play a role for the cell. In the laboratory, however, mutant *Escherichia coli* lacking alkaline phosphatase survive quite well, as do mutants unable to shut off alkaline phosphatase production.

Use in research

The most common alkaline phosphatases used in research are:

- Bacterial alkaline phosphatase (BAP), from *Escherichia coli* C4 cells
- Shrimp alkaline phosphatase (SAP), from a species of arctic shrimp (*Pandalus borealis*)
- Calf intestine alkaline phosphatase (CIAP), from calf intestine
- Placental alkaline phosphatase (PLAP) and its C terminally truncated version that lacks the last 24 amino acids (constituting the transmembrane domain) - the secreted alkaline phosphatase (SEAP)

Alkaline phosphatase has become a useful tool in molecular biology laboratories, since DNA normally possesses phosphate groups on the 5' end. Removing these phosphates prevents the DNA from ligating (the 5' end attaching to the 3' end of another molecule), thereby preventing DNA degradation until the next step of the process for which it is being prepared; also, removal of the phosphate groups allows radiolabeling (replacement by radioactive phosphate groups) in order to measure the presence of the labeled DNA through further steps in the process or experiment. For these purposes, the alkaline phosphatase from shrimp is the most useful, as it is the easiest to inactivate once it has done its job.

Another important use of alkaline phosphatase is as a label for enzyme immunoassays.

One common use in the dairy industry is as a marker of pasteurisation. This molecule is denatured by elevated temperatures found during pasteurisation, and can be tested for via colour change of a para-nitro-phenol phosphate substrate in a buffered solution (Aschaffenburg Mullen Test). Raw milk would typically produce a yellow colouration within a couple of minutes, whereas properly pasteurised milk should show no change. There are of course exceptions to this in the case of heat stable alkaline phosphatases produced by some bacteria.

Inhibitors

All mammalian alkaline phosphatase isoenzymes except placental (PLAP and SEAP) are inhibited by homoarginine and similarly all except the intestinal and placental ones are blocked by levamisole. Heating for ~2 hours at 65°C inactivated most isoenzymes except Placental isoforms (PLAP and SEAP).

Human Physiology

In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone, and the placenta. The optimal pH for the enzyme activity is $\text{pH}=10$ ^[citation needed] in standard conditions (298K, 1 atm).

Diagnostic use

High ALP levels can show that the bile ducts are blocked.^[1] Blood plasma (serum) levels of ALP are typically 20-70 units per liter in adults (Reference - USMLE),^[citation needed] depending on the assay and local normal guidelines. Levels are significantly higher in children and pregnant women.

Lowered levels of ALP are less common than elevated levels.

The following conditions can cause abnormal levels of ALP:

Elevated levels (hyperphosphatasemia)

If it is unclear why alkaline phosphatase is elevated, isoenzyme studies using electrophoresis can confirm the source of the ALP. Heat stability also distinguishes bone and liver isoenzymes ("bone burns, liver lasts").

- Liver (Liver ALP):
 - Cholestasis, cholecystitis, cholangitis, cirrhosis, hepatitis, fatty liver, liver tumor, liver metastases, drug intoxication
 - N.B. concurrently elevated GGT (gamma glutamyl transpeptidase) helps rule in favor of liver metastasis (rather than bone, kidney, etc.) when assessing spread of cancer.
 - Drugs: e.g. verapamil, carbamazepine, phenytoin, erythromycin, allopurinol, ranitidine
- Bone disease (Bone ALP):
 - Paget's disease, osteosarcoma, bone metastases of prostatic cancer (High / very high ALP values)
 - Other bone metastases
 - Fractured bone
 - Multiple myeloma (only when associated with fractures)
- Skeletal involvement of other primary diseases:
 - Osteomalacia, rickets, vitamin D deficiency, (Moderate rise)
 - Malignant tumors (ALP originating from tumor)
 - Renal disease (secondary hyperparathyroidism)
 - Primary hypothyroidism
- Polycythemia vera
- Myelofibrosis
- Leukemoid reaction to infection
- Women using hormonal contraception
- Pregnancy
- Biliary obstruction
- Transient hyperphosphatasemia of infancy: benign, often associated with infection

Lowered levels (hypophosphatasemia)

- Hypophosphatasia, an autosomal recessive disease
- Postmenopausal women receiving estrogen therapy because of osteoporosis
- Men with recent heart surgery, malnutrition, magnesium deficiency, hypothyroidism or severe anemia
- Children with achondroplasia and cretinism
- Children after a severe enteritis
- Pernicious anemia
- Aplastic anemia
- Chronic myelogenous leukemia

Other notes

Leukocyte alkaline phosphatase (LAP) is found within white blood cells. Blood levels of LAP can help in the diagnosis of chronic myelogenous leukemia (CML) and leukemoid reaction.

Key Terms

Alkaline phosphatase

An enzyme found throughout the body, primarily in liver, bone, placenta, and intestine.

Cholestasis

Stoppage or suppression of the flow of bile.

Enzyme

A substance needed to trigger specific chemical reactions.

Hepatocellular

Of or pertaining to liver cells.

Hepatocyte

A liver cell.

Isoenzyme

A variation of an enzyme

Use

Causes of high alkaline phosphatase include bone growth, healing fracture, acromegaly, osteogenic sarcoma, liver or bone metastases, leukemia, myelofibrosis, and rarely myeloma. Alkaline phosphatase is used as a tumor marker.^{1,2}

In rickets and osteomalacia, serum calcium and phosphorus are low to normal, and alkaline phosphatase may be normal or increased.

Hypervitaminosis D may cause elevations in alkaline phosphatase.

In Paget disease of bone there is often isolated elevation of serum alkaline phosphatase. Some of the highest levels of serum ALP are seen in Paget disease.

Hyperthyroidism, by its effects upon bone, may elevate alkaline phosphatase. There is evidence that thyroid hormone (T_3) acts to stimulate bone alkaline phosphatase activity through an osteoblast nuclear receptor-mediated process. Hyperparathyroidism, in some patients. Pseudo hyperparathyroidism.

Chronic alcohol ingestion (in chronic alcoholism, alkaline phosphatase may be normal or increased, but often with high AST (SGOT) and/or high bilirubin and especially with high GGT; MCV may be high).

Biliary obstruction (tenfold increase may be seen with carcinoma of the head of pancreas, choledocholithiasis); cholestasis; GGT also high. Cholecystitis with cholangitis. (In most patients with cholecystitis and cholangitis who do not have a common duct stone, alkaline phosphatase is within normal limits or only slightly increased.) Sclerosing cholangitis (eg, with ulcerative colitis), although importantly, 3% of cases of symptomatic sclerosing cholangitis may have normal serum ALP. Endoscopic retrograde cholangiography might be considered then in patients with diseases known to be associated with primary sclerosing cholangitis and with appropriate symptomatology even though ALP level is normal.

Primary or metastatic tumor in liver: there may be marked increase

and GGT is often high. Only three laboratory markers were consistently abnormal, in evaluating for metastatic carcinoma of breast, prior to clinical detectability of metastases: these were alkaline phosphatase, GGT and CEA.

Cirrhosis, especially in primary biliary cirrhosis, in which fivefold or more increases are seen.

Gilbert syndrome: Increase in intestinal alkaline phosphatase is seen.

Hepatitis: Moderate increases in alkaline phosphatase occur in viral hepatitis, but greater elevations of the transaminases (AST (SGOT), ALT (SGPT)) are usually found.

Fatty metamorphosis of liver (moderate increase occurs in acute fatty liver).

Diabetes mellitus, diabetic hepatic lipidosis.

Infiltrative liver diseases (eg, sarcoid, TB, amyloidosis, abscess).

Sepsis. Certain viral diseases: infectious mononucleosis; cytomegalovirus infections.

Postoperative cholestasis. Pancreatitis, carcinoma of pancreas, cystic fibrosis.

Pulmonary infarct (1-3 weeks after embolism. Healing infarcts in other organs, including kidney, may also cause increased alkaline phosphatase); other situations in which angiofibroplasia occurs, such as healing in a large decubitus ulcer.

Tumors, especially hypernephroma; neoplastic ectopic production (Regan, Nagao isoenzymes).

Fanconi syndrome.

Peptic ulcer, erosion. Intestinal strangulation or obstruction, or

ulcerative lesion. Steatorrhea, malabsorption (from bone, secondary to vitamin D deficiency). Ulcerative colitis with pericholangitis, other erosive lesions of colon.

Congestive heart failure.

Parenteral hyperalimentation of glucose, intravenous albumin administration.

Familial hyperphosphatasemia.

Idiopathic.

Drugs - estrogens (large doses), birth control agents, methyltestosterone, phenothiazines, oral hypoglycemic agents, erythromycin, or any drug producing hypersensitivity or toxic cholestasis. Many commonly and uncommonly used drugs elevate alkaline phosphatase, and tenfold increase may be seen with drug cholestasis.

Causes of low alkaline phosphatase are said to include:

Hypothyroidism - but most hypothyroid patients have normal alkaline phosphatase.

Pernicious anemia - in very few patients.

Hypophosphatasia: Very low alkaline phosphatase values are found in the presence of normocalcemia or hypocalcemia. This diagnosis may be confirmed by quantitation of urinary phosphoethanolamine.

Malnutrition has been reported to relate to low values, but in practice, diseases causing malnutrition relate often to high alkaline phosphatase results (eg, disseminated neoplasia).

Some drugs (clofibrate, azathioprine, estrogens and estrogens in combination with androgens) lower serum ALP activity.

Limitations

Used alone, alkaline phosphatase may be misleading.

Methodology

Kinetic

Additional Information

Serum alkaline phosphatase is a member of a family of zinc metalloprotein enzymes that function to split off a terminal phosphate group from an organic phosphate ester. This enzyme functions in an alkaline environment (optimum pH of 10). Active center of ALP enzymes includes a serine residue. Mg and Zn ions are required for minimal activity. Enzyme activity is localized in the brush border of the proximal convoluted tubule of the kidney, intestinal mucosal epithelial cells, hepatic sinusoidal membranes, vascular endothelial cells and osteoblasts of bone. There are distinctive forms of ALP in the placenta and small intestine; hepatic, renal and osteoblast (bone) ALP are similar molecules.

Serum ALP activity of intestinal origin occurs only in individuals of ABO blood type O or A. They are secretors of ABH RBC antigens and also carry the Lewis red cell antigen. Serum intestinal ALP level increases in these individuals about 2 hours following consumption of a fatty meal.

Liver alkaline phosphatase is increased in cholestasis and inflammatory liver disease as well as in infiltrative liver disease. The enzyme is sensitive to obstructive biliary processes, even small secondary bile duct obstruction, and thus may be increased in those patients when the bilirubin is normal due to compensatory bilirubin excretion by the rest of the liver. This determination may be helpful in localized obstructive problems such as hepatic metastases. An electrophoretically slow moving isoenzyme with high relative mass may occur in some patients with bile duct obstruction and hepatic metastases and may result in false elevation of CK-MB.

To confirm biliary abnormality, an additional useful test is GGT. GGT is elevated in hepatobiliary disease, not in uncomplicated bone

disease.

Serum ALP is increased during pregnancy. Marked decline of high ALP of pregnancy is seen with placental insufficiency and imminent fetal demise