Laboratory Personnel

As well as performing the analyses, the clinical biochemistry laboratory also provides a consultative service. The laboratory has on its staff both medical and scientific personnel who are familiar with the clinical significance and the analytical performance of the test procedures, and they will readily give advice on the interpretation of the results. Do not be hesitant to take advantage of this advice, especially where a case is not straightforward. Every biochemistry analysis should attempt to answer a question which the clinician has posed about the patient.

Specimen collection:

In order to carry out biochemical analyses, it is necessary that the laboratory be provided with both the correct specimen for the requested test, and also information which will ensure that the right test is carried out and the result returned to the requesting clinician with the minimum of delay. As much detail as possible should be included on the request form to help both laboratory staff and the clinician in the interpretation of results. This information can be very valuable when assessing a patient's progress over a period, or reassessing a diagnosis. Patient identification must be correct, and the request form should include some indication of the suspected pathology. The requested analyses should be clearly indicated. Request forms differ in design.

A variety of specimens are used in biochemical analysis and these are shown in table 1. However, the majority of biochemical tests are performed on serum from venous blood or urine.

Venous blood, serum or plasma
Arterial blood
Capillary blood

Urine
Faeces
Cerebrospinal fluid
Sputum and saliva
Tissue and cells
Calculi (stones)

Blood specimens

If blood is collected into a plain tube and allowed to clot, after centrifugation a serum specimen is obtained. For many biochemical analyses this will be the specimen recommended. In other cases, especially when the analyte in question is unstable and speed is necessary to obtain a specimen which can be frozen quickly, the blood is collected into a tube containing an anticoagulant such as heparin. When centrifuged, the supernatant is called plasma which almost identical to the cell- free fraction of blood but contains the anticoagulant as well.

Urine specimens:

Urine specimen containers may include a preservative to inhibit bacterial growth, or acid to stabilize certain metabolites. They need to be large enough to hold a full 24h collection. Random urine samples are collected into small 'universal' containers.

Other specimen types:

For some tests, specific body fluids or tissue may be required. There will be specific protocols for the handling and transport of these samples to the laboratory.

Dangerous specimen:

All specimens from patients with dangerous infections should be labelled with a yellow "dangerous specimen" sticker. A similar should be attached to request form. Of most concern to the laboratory staffs are hepatitis B and HIV, but all specimens should always be treated both by clinicians and biochemistry staff as potentially hazardous.

Sampling Errors

There are a number of potential errors which may contribute to the success or failure of the laboratory to provide the correct answers to the clinician's questions. Some of these problems arise when a clinician first obtains specimens from patient.

- Blood sampling technique. Difficulty in obtaining a blood specimen may lead to haemolysis with consequent release of potassium and other red cell constituents. Results for these will be falsely elevated.
- Prolonged stasis during venepuncture. Plasma water diffuses into the interstitial space and the serum or plasma sample obtained will be concentrated. Proteins and protein-bound components of plasma such as calcium or thyroxine will be falsely elevated.
- Insufficient specimen. Each biochemical analysis requires a certain volume of specimen to enable the test to be carried out. It may prove to be impossible for the laboratory to measure everything requested on a small volume specimen.
- Errors in timing. The biggest source of error in the measurement of an analyte in a 24-hour urine specimen is in the collection of an accurately timed volume of urine.
- Incorrect specimen container. For many analyses the blood must be collected into a container with anticoagulant and preservative. For example, samples for glucose should be collected into a special container containing fluoride which

inhibits glycolysis; otherwise the time taken to deliver the sample to the laboratory can affect the result. If a sample is collected into the wrong container, it should never be decanted into another type of tube. For example, blood which has been exposed even briefly to EDTA (an anticoagulant used in sample containers for lipids) will have a markedly reduced calcium concentration, approaching zero.

Abnormal appearances of specimens

Any abnormal appearance of a specimen should be reported and investigated if indicated. Such action on the part of a technician may lead to a condition being diagnosed more rapidly and a patient receiving appropriate treatment at an earlier stage. The following are examples of abnormal specimen appearances and their possible significance:

- A dark brown serum may indicate intravascular haemolysis due to sickle cell disease, severe malaria, or an incompatible blood transfusion.
- A lipaemic (fatty) serum is associated with raised triglycerides (above 3.4 mmol/l).
- A deep yellow (icteric) serum indicates that a patient is jaundiced.
- A serum sample that is abnormally viscous (thick) or turbid may contain paraproteins.
- A serum that becomes markedly turbid after being refrigerated may contain cryoglobulins or cold agglutinins.
- A blood sample that contains a high concentration of red cells from which little serum or plasma can be obtained indicates severe dehydration or a blood disorder.

Enzymes

Serum enzymes in disease

Enzymes may be classified into two groups. Some, such as the enzymes of the coagulation cascade, have a defined function in blood. Others were originally cell constituents, or were made within cells to be secreted into the gastrointestinal tract. They appear in the blood incidentally and their measurement is of value in diagnosis. Damage to the tissues of origin, or proliferation of the cells from which these enzymes arise, will lead to an increase in the activity of these enzymes in plasma. It should be noted that increase in serum enzyme activity are only roughly proportional to the extent of tissue damage.

Enzymes that have been shown to have a diagnostic value are:

- **Acid phosphatase:** a tumor marker in prostatic carcinoma.
- **Alanine aminotransferase (ALT):** an indicator of hepatocellular damage.
- **Aspartate aminotransferase (AST):** an indicator of hepatocellular damage, or as a marker of muscle damage, such as a myocardial infarction (MI).
- **Alkaline phosphatase:** increase in cholestatic liver disease and is a marker of osteoblast activity in bone disease.
- **γ-glutamyl transferase:** a sensitive marker of liver cell damage.
- **Creatine Kinase:** a marker of muscle damage and acute MI.
- **Lactate dehydrogenase:** a marker of muscle damage.
- **Amylase:** an indicator of cell damage in acute pancreatitis.

Isoenzyme determination

Some enzymes are present in the plasma in two or more molecular form. These variants are known as isoenzymes, and although they have different structures they

perform the same catalytic function. Different isoenzymes may arise from different tissues and their specific detection may give clues to the site of pathology.

Alkaline phosphatase isoenzymes may distinguish between bone and liver disease, especially in patients in whom metastases of bone or liver are suspected. A specific isoenzyme of creatine kinase (CK-MB) is useful in the early detection of myocardial infarction. Heart muscle contains proportionally more of this isoenzyme than skeletal muscle, and raised levels of CK-MB indicate that a myocardial infarction has occurred.

Principle of enzyme determination

Enzymes are usually present in very small amounts in plasma or serum, therefore they cannot be measured directly like other substances. They are assayed indirectly by measuring either the reduction in concentration of the substrate upon which the enzyme acts or the amount of product formed from the substrate. It is therefore enzyme activity that is being measured.

Determination of aminotransferases

- The aminotransferases are aspartate aminotransferase (AST), previously known as glutamate oxaloacetate transaminase (GOT), and alanine aminotransferase (ALT), formerly known as glutamate pyruvate transaminase (GPT), are concerned with amino acid metabolism.
- Alanine transaminase (ALT) and Aspartate transaminase (AST) enzymes are the most abundantly present in the liver and is elevated in blood as a result of leakage from damaged cells.
- Measurement of these transaminases is useful for the diagnosis of liver diseases
- In viral hepatitis the enzyme levels are increased 20-50 times above the upper limit of the normal range.
- AST is present in both mitochondria and cytosol of cells, while ALT is found in cytosol only.
- These enzymes catalyze transfere of alpha-amino groups of alanine and aspartate respectively to the alpha-keto group of alpha-ketoglutaric acid.

Pyruvate + glutamate $\xrightarrow{\textbf{ALT}}$ alanine + alpha-ketoglutatrate.

Oxaloacetate + glutamate $\xrightarrow{\textbf{AST}}$ aspartate + alpha-ketoglutatrate.

Measurement of serum or plasma alanine aminotransferase (ALT) activity

Analyte

- Alanine aminotransferase (ALT), also called serum glutamic pyruvic transaminase (SGPT).
- ALT catalyzes the transfere of alpha-amino group of alanine to alpha-ketoglutaric acid resulting in the formation of pyruvic and glutamic acid.
- ALT is found primarily in liver
- ALT is found in greatest concentration within the cytoplasm of liver tissues.
- ALT is found in lesser activity in the skeletal muscle, kidneys, heart, pancrease, spleen, lungs and red blood cells (RBCs).
- The enzyme half life is 2-5 days following acute hepatic injury, serum enzyme activity peak at 48 hours and then begins to decrease.

Value of test

Measurement of ALT activity is mainly performed to investigate liver disease. Increasingly ALT is being measured to monitor patients receving antiretroviral drugs associated with hepatotoxicity.

Principle (kinetic method):

The pyruvate formed in the first reaction is reduced to lactate in the presence of lactate dehydrogenase and NADH. The activity of ALT is determined by measuring the rate of oxidation of NADH at 340 nm.

α-oxaloglutarate + L-alanine $\xrightarrow{\text{ALT}}$ L-gultamate + pyruvate

pyruvate + NADH+H $\xrightarrow{\text{LDH}}$ L-lactate + NAD^+

Measurement of serum or plasma aspartate aminotransferase (AST) activity

Analyte

- Aspartate aminotransferase (AST) also called serum glutamic oxaloacetic transaminase (SGOT).
- AST catalyzes the transfere of alpha-amino group of aspartate to alpha-ketoglutaric acid resulting in the formation of oxaloacetic acid and glutamic acid.
- AST is not organ specific. skeletal muscle contains the highest concentration followed by liver and cardiac muscle.
- Large amounts of AST are present in the liver, kidneys, cardiac muscle, and skeletal muscle. Small amounts of the enzyme are present in the brain, pancreas, and lungs.
- The enzyme half life is 5-12 hours.
- When there is liver cell damage the serum or plasma levels of both enzymes are raised.

Value of test

Increasingly AST is being measured to monitor patients suffer from heart disease. While both ALT and AST are raised with hepatocellular injury, ALT is more specific for detecting liver cell damage.

Principle (kinetic method):

α-oxoglutarate reacts with L-aspartate in the presence of AST to form L-glutamate plus oxaloacetate. The indicator reaction utilizes the oxaloacetate for a kinetic determination of NADH consumption at 340nm.

α-oxoglutarate + L-aspartate —— AST ⟶ L-glutamate + oxaloacetate

Oxaloacetate + $NADH+H^+$ —— MDH ⟶ L-malate + NAD^+

Clinical significance of ALT and AST

The activities of both AST and ALT are high in tissues especially liver, heart, and muscles.

Any damage or injury to the cells of these tissues may cause release of these enzymes alongwith other intracellular proteins/enzymes into the circulation leading to increase activities ofthese enzymes in the blood. Some increases in the activities of both the enzymes are seen after alcohol intake.

Liver diseases

Determinations of activities of AST and ALT in serum in patients with liverdiseases like viral hepatitis and other forms of liver diseases with necrosis, give high valueseven before the appearance of clinical signs and symptoms like jaundice. Activity levelsof20 to 50 fold higher than normal are frequently seen in liver cells damage but it may reach ashigh as 100 times in severe damage to cells. Highest serum activities are seen between 7th and12th days and return to normal levels by the 3rd to 5th week. In sever tissue damage ALTactivity is higher than AST and the ALT:AST ratio becomes ≥1(normally<1). In cirrhosis the level ofactivities vary with the severity of the disease. It may increase only up to 5 fold of the normalactivities. Up to 10 fold increase is seen in carcinoma of the liver. Even though the activities ofboth AST and ALT are elevated in the serum of the patients with liver diseases, ALT is moreliver specific enzyme as increased ALT activity in serum is hardly seen in tissues other than liver cell damage.

Heart diseases

In myocardial infarction high activity of AST is seen in serum. ALT activityis within normal range or slightly increased in uncomplicated myocardial infarction. Rise inAST is seen within 6 to 8 hours of the onset of chest pain, highest level at 18 to 24 hours andreturns to preinfarction levels by 4th to 5th day. There are other superior markers available formyocardial infarction as AST lacks the tissue specific characteristics, as its activity may alsoincreased in diseases of other tissues like liver and skeletal muscles.

Skeletal muscle diseases

AST and occasionally ALT activity levels are increased inprogressive muscular dystrophy and dermatomyositis. Level of AST may go as high as 8times of the normal. There is no increase in the enzyme activity in the muscle diseases ofneurogenic origin. Increased AST activity, 2 to 5 times of normal, is also seen after crushedmuscle injuries.

In other conditions like pulmonary emboli, acute pancreatitis, hemolytic disease andgangrene the activity of AST is found to be 2 to 5 times higher than the normal activity.

Measurement of serum or plasma alkaline phosphatase (ALP) activity

Analyte

Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment.

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. Humans and most other mammals contain the following alkaline phosphatase isozymes:

- ALPI – intestinal
- ALPL – tissue non-specific (liver/bone/kidney)
- ALPP – placental (Regan isozyme)

Value of test

The alkaline phosphatase test (ALP) is used to help detect liver disease or bone disorders. In conditions affecting the liver, damaged liver cells release increased amounts of ALP into the blood. This test is often used to detect blocked bile ducts because ALP is especially high in the edges of cells that join to form bile ducts. If one or more of them are obstructed, for example by a tumor, then blood levels of ALP will often be high.

Any condition that affects bone growth or causes increased activity of bone cells can affect ALP levels in the blood. An ALP test may be used, for example, to

detect cancers that have spread to the bone or to help diagnose Paget's disease. This test may also sometimes be used to monitor treatment of Paget's disease or other bone conditions, such as vitamin D deficiency.

Principle (kinetic method):

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of phosphate group from 4-nitrophenylphosphate to 2- amino-2-methyl-1-propanol (AMP) liberating 4- nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405nm.

4-nitrophenylphosphate + AMP $\xrightarrow{\text{ALP}}$ AMP-phosphate + 4- nitrophenol

Clinical significance

Physiological bone growth elevates ALP in serum and hence in the sera of growing children enzyme activity is 1.5-2.5 times that in normal adult serum. The level of ALP in the serum of women in the third trimester of pregnancy is 2-3 times more than that of normal level.

Liver diseases

Biliary obstruction due to any cause may elevate ALP level by increasing its synthesis from the hepatocytes adjacent to the biliary canaliculi. This newly synthesized ALP enters the circulation and elevates the enzyme level in the serum. Elevation of ALP in the serum is more with extrahepatic obstruction by stones or by carcinoma head of pancreas than in intrahepatic obstruction. The enzyme level may return to normal on removal of the obstruction. Liver diseases affecting parenchymal cells like infectious hepatitis show only moderate elevation or normal serum ALP levels.

Bone diseases

Bone diseases with increased osteoblastic activity shows increased ALP level in the serum. High ALP levels sometimes up to 25 times the normal value are seen in osteitisdeformans (Paget's disease). In Paget's disease there is resorption of bones due to uncontrolled osteoclastic activity and body tries to rebuild bone by increasing osteoblastic activity leading to high ALP level. Moderate increase in ALP level in the serum is seen in osteomalacia, rickets (comes down to normal on treatment with vitamin D), Fanconi's syndrome, primary and secondary hyperparathyroidism.Secondaries in bone from prostate

Cancer

show high serum ALP. Very high ALP levels are present in patients with osteogenicbone cancer.

Measurement of serum or plasma gamma-glutamyl transferase (GGT) activity

Analyte

GGT is an enzyme found in many organs, such as the kidney, liver, spleen and pancreas; however, the main source of GGT in the blood is the liver. GGT catalyzes the transfer of the gamma-glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate).

Value of test

GGT is increased in most diseases that cause acute damage to the liver or bile ducts but is usually not helpful in distinguishing between different causes of liver damage. For this reason, use of GGT is controversial. It was suggested that it can be useful in determining the cause of a high alkaline phosphatase (ALP), another liver enzyme.

Principle (kinetic method):

The rate of liberation of yellow coloured indicator 5-amino-2-nitrobenzoate is directly proportional to γ-Glutamyl activity in the sample and is quantitated by measuring the increase in absorbance at 405 nm.

L-γ-Glutamyl-3-carboxy-4 -nitroanilide + Glycylglycine

↓ **GGT**

L-γ-Glutamyl- glycylglycine + 5-amino-2-nitrobenzoate

Clinical significance

Liver diseases

The activity of GGT in serum is elevated in all forms of liver disease.Highest activity is seen in cases of intra- and post-hepatic biliary obstruction. GGT is moresensitive than ALP in detecting obstructive jaundice, cholangitis and cholecystitis. In infectious hepatitis there is only moderate elevation of GGT activity in the serum. In cases of primary and secondary liver cancer the level of GGT increase earlier and more pronounced than other enzymes of the liver. Increased levels of GGT are seen in heavy drinkers and patients with alcoholic liver cirrhosis. High levels are also found in patients receivinganticonvulsant drugs. Increased levels of GGT are found in almost all the diseases involvingliver but are of little value in discriminating different kinds of liver disease. Normal levels of GGT are seen in patients with muscle diseases, children older than 1 year or in healthy pregnant women – conditions in which ALP is elevated. Thus measurement of GGT levels in serum can be used to ascertain whether observed elevations of ALP are due to skeletal disease or reflect the presence of hepatobiliary disease.

Pancreatic diseases

GGT activity may increase in acute as well as chronic pancreatitis and in some cases of pancreatic malignancies especially if associated with hepatobiliaryobstruction.

Tumors

Prostate malignancy may at time show increased level of GGT in serum. The irradiation of tumors in cancer patients may be accompanied by a rise in GGT

activity. In general if there is increased GGT level in serum of cancer patients there is a chance that the tumor might have metastasize to the liver.

Measurement of serum or plasma lactate dehydrogenase (LDH) activity

Analyte

The enzyme, lactate dehydrogenase(also called lactic acid dehydrogenase, or LDH) is an enzyme found in almost all body tissues .It plays an important role in cellular respiration, the process by which glucose (sugar) from food is converted into usable energy for our cells. LDH is present in many kinds of organs and tissues throughout the body, including the liver, heart, pancreas, kidneys, skeletal muscles, brain, and blood cells.

When illness or injury damages your cells, LDH may be released into the bloodstream, causing the level of LDH in your blood to rise. High levels of LDH in the blood indicate acute or chronic cell damage, but additional tests will be necessary to discover its cause. Abnormally low LDH levels occur only rarely and are not usually harmful.

Types of LDH

There are five different forms of LDH, and they are distinguished by slight differences in their structure. Each form of the LDH enzyme is called an isoenzyme. The isoenzymes of LDH are LDH-1, LDH-2, LDH-3, LDH-4, and LDH-5.

Different LDH isoenzymes are found in different body tissues. The areas of highest concentration for each type of isoenzyme are listed below:

LDH-1: heart and red blood cells

LDH-2: white blood cells

LDH-3: lungs

LDH-4: kidneys, placenta, and pancreas

LDH-5: liver and skeletal muscle

Value of test

The LDH test is generally used to screen for tissue damage. This damage may be acute (as in the case of a traumatic injury) or chronic (due to a long-term condition such as liver disease or certain types of anemia). It also may be used to monitor progressive conditions, such as muscular dystrophy and HIV. Even though an LDH test is useful in diagnosing tissue damage, other tests are usually necessary to pinpoint the location of the damage. One such test is called the LDH isoenzymes test. LDH isoenzymes are five kinds of the LDH enzyme that are found in specific concentrations in different organs and tissues. By measuring the blood levels of these isoenzymes, doctors can get a better idea of the type, location, and severity of the cellular damage.

Principle (kinetic method):

Kinetic determination of LDH activity is according to the following reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\textbf{LDH}} \text{L-Lactate} + NAD^+$$

Clinical significance

Heart diseases

In myocardial infarction the level of total LDH activity in serum is 3-4 timesthat of normal but it may go up to 10 times the normal value. In myocarditis and cardiacfailure with hepatic congestion the serum enzyme activity may be elevated. Moderateelevation of the enzyme activity may be seen in severe shock

and anoxia. But no change inserum enzyme activity in angina and in pericarditis. Hemolysis due to any cause may elevatelevel of LDH1 & 2 in the serum.

Liver diseases

In toxic hepatitis with jaundice serum activity of LDH may be elevated upto10 times the normal value. Increased activity in serum is also seen in viral hepatitis. SerumLDH5 activity is often elevated in patients with primary liver disease and liver anoxiasecondary to decreased O2 perfusion.

Muscle diseases

The patients with progressive muscular dystrophy often show elevatedLDH activity especially LDH5 isoenzyme in the serum. In the later stages of the diseasewhen the muscle mass is lost the LDH level may come down to normal level.

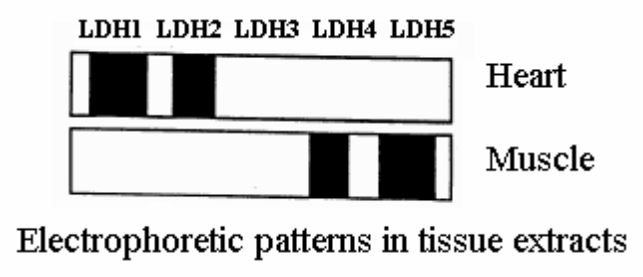
Kidney diseases

In chronic glomerulonephritis,systemic lupus erythematosus, diabeticnephrosclerosis and bladder & kidney malignancies the LDH activity in the urine is found tobe elevated 3 to 6 times the normal.

Tumors

Patients with malignant disease show increased LDH activity in serum especiallyLDH4 & 5. In germ cell tumors like teratomas, seminoma of the testis high level of LDH1 isseen.

Note: The efficiency of the conversion of pyruvate tolactate increases with the number of M chains.



Electrophoretic patterns in tissue extracts

What are the metabolic consequences for heart and muscles?

The high concentration of LDH 5 (4 M subunits) in skeletal muscle rapidly converts pyruvate to lactate,while the high concentration of LDH 1 (4 H subunits) in heart tissue favorsconversion of pyruvate to acetyl CoA which enters the citric acid cycle.

In skeletal muscle, where oxygen deprivation is common during exercise, the reaction is efficient and large amounts of lactatecan be formed.

In tissues that preferentially oxidize glucose aerobically to CO2and water, such as cardiac muscle, the reaction is not efficientand pyruvate is preferentially converted to acetyl CoA whichenters the citric acid cycle.

Measurement of serum or plasma creatine kinase (CK) and creatine kinase-MB(CK-MB) activity

Analyte

Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phospho-creatine kinase (and sometimes incorrectly as **creatinine** kinase), is an enzyme expressed by various tissues and cell types. CK catalyses the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). This CK enzyme reaction is reversible and thus ATP can be generated from PCr and ADP. It occurs as three different isoenzymes, each composed of two polypeptide chains, B and M. skeletal muscle has a very high CK content; usually, 98% CK-MM and 2% CK-MB. cardiac muscle also has a very high CK content; usually, 70-80% CK-MM and 20-30% CK-MB. Brain, prostate, thyroid, gut and lung has predominantly CK-BB. plasma has predominantly CK-MM with less than 6% CK-MB. usually, the heart is the only tissue in which the amount of CK-MB exceeds 5%. Exceptions are patients with muscle disease and in athletes, in which the skeletal muscle content of CK-MB may rise to 5-15%.Normally the laboratory provides total CK levels. Normal ranges are 24-170 Units/litre in women, 24-195 in men. A heparinised plasma is required.

Value of test

creatine kinase levels in the blood are measured as part diagnosis of a number of illnesses. These include acute renal failure (breakdown of kidney function), myocardial infarction (heart attack), muscular dystrophy, and rhabdomyolysis (severe muscle breakdown).

a test for creatine kinase is indicated whenever a patient suffers from symptoms of heart attack, acute renal failure, or muscular breakdown .

The symptoms of a heart attack include chest pains, difficulty breathing or shortness of breath, sleep disturbance, weakness, and lack of energy .

The symptoms of acute renal failure include decreased urine production, swelling or inflammation in parts of the body, difficulty concentrating or confusion, fatigue and lethargy, nausea, vomiting and diarrhea .The symptoms of muscular breakdown such as muscular dystrophy are primarily a weakness of the muscles themselves.

Principle of CK (kinetic method):

Creatine kinase catalyzes the phosphorylation of ADP in the presence of creatine kinase to form ATP and creatine. The catalytic concentrationis determined from the rate of NADPH formation measured at 340nm by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G_6PDH) coupled reaction 1,2.

Creatine Phosphate + ADP $\xrightarrow{\textbf{CPK}}$ Creatine + ATP

ATP + Glucose $\xrightarrow{\textbf{HK}}$ ADP + glucose-6-phosphate

Glucose-6-phosphate + $NADP^+$ $\xrightarrow{\textbf{G6PDH}}$ 6-Phosphogluconate + NADPH $+H^+$.

Principle of CK-MB (kinetic method):

A specific antibody inhibits the M subunits of CK-MM and CK-MB and thus allows determination of B subunit of CK-MB (assuming the absence of CK-BB or CK-1). CK-B catalytic concentration, which corresponds to half of CK-MB concentration, is determined from the raye of NADPH formation,

measured at 340 nm, by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G_6PDH) coupled reaction 1, 3.

Creatine Phosphate + ADP —**CPK**→ Creatine + ATP

ATP + Glucose —**HK**→ ADP + glucose-6-phosphate

Glucose-6-phosphate + $NADP^+$ — **G6PDH** → 6-Phosphogluconate + NADPH $+H^+$.

Clinical significance

Serum CK activity is elevated in tissue damages involving skeletal muscle, heart muscle,brain injury etc. Elevation of particular CK isoenzymes activity in serum is of diagnosticvalue.

Heart diseases

CK activity in serum invariably increases after myocardial infarction (MI). CK-2 levels rise 3 to 6 hours after a heart attack. If there is no further damage to the heartmuscle, the level peaks at 12 to 24 hours and returns topreinfarction level in 12 to 48 hours.The use of total CK and CK-2 in the diagnosis of myocardial infarction is the most importantsingle application of CK measurements in clinical chemistry. Percentage of CK-2 over totalCK activity is valuable in diagnosis of MI. Preinfarction values of CK-2 are usually less than6% of the total CK activity, but following an infarction values can increase up to 30%depending on the extent of myocardial damage, location of the infarct or the methods used foranalysis.

The diagnostic sensitivity of total CK in myocardial infarction is 93-98% and for CK-2 it isnearly 100%. And the diagnostic specificity is 75-85% for total CK and nearly 100% for CK-2 if the level of CK-2 is >6% of total CK.

Elevation of total CK and CK-2 is seen in cardiac trauma following heart surgery which maymask elevation due to intraoperative myocardial infarction. Other cardiac conditions likeangina pectoris, cardiogenic shock, electrical counter shock, myocarditis, congestive heartfailure, cardiac intra arterial procedures have reportedly shown elevations of total serum CKor CK-2 or both. CK-2 level of <5% or 6% of total CK usually exclude the myocardialdamage.

Skeletal muscle diseases

High CK activity is found in all types of muscular dystrophy. Incase of Duchenne muscular dystrophy the level may go as high as 50 times that of the normal level. In progressive muscular dystrophy, enzyme activity in serum is highest in childhoodand may be elevated long before the disease is clinically apparent, About 50 to 80% of asymptomatic female carriers of Duchenne dystrophy show 3-6 fold elevations of CK activity. Elevation of CK activity is observed in other muscular diseases including malignant hyperthermia. Muscle disease of neurogenic origin may have normal CK activity in the serum.

CNS diseases

In cerebral ischemia , acute cerebro vascular disease, head injury and neurological interventions the level of CK in the serum may increase. In Reye's syndrome, which is characterized by acute brain swelling with fatty infiltration, the CK activity may riseto upto 70 fold of normal level.

Thyroid diseases

There is an inverse relationship with thyroid activity. Elevation of CK activity upto 5 times the normal level is seen in about 60% of the hypothyroid subjects. The major enzyme present is CK-3, CK-2 may go up to 13% of the total CK activity if myocardial involment is there in the hypothyroid subjects.

Measurement of serum or plasma alpha amylase activity

Analyte

The enzyme alpha-amylase is present in large amounts in pancreatic juice and saliva. Pancreatic amylase hydrolyzes starches not split by salivary amylase. Some of the pancreatic amylase is absorbed into the blood and is excreted in the urine. Acute inflammation of the pancreas (pancreatitis) causes the release of large amounts of amylase into the circulation.

Value of test

Measurement of serum or plasma amylase activity is usually requested to assist in the differentiation of acute pancreatitis from other acute abdominal disorders. It is an early indicator of acute pancreatitis.

Principle of AMS:

Alpha-amylase hydrolyzes the 2-chloro-4-nitrophenyl-α-maltotrioside (CNPG3) to release 2-chloro-4-nitrophenol (CNP) and form 2-chloro-4-nitrophenyl-α-maltoside (CNPG2), maltotriose (G3) and glucose (G). the rate of formation of the 2-chloro-4-nitrophenol can be detected at 405nm to give a direct measurement of alpha-amylase activity in the sample.

$$5\ \text{CNPG3} \xrightarrow[\textbf{AMS}]{} 3\text{CNP} + 2\text{CNPG2} + 3\text{G3} + 2\text{G}$$

Clinical significance

Pancreas

Amylase activity assay in serum and urine are mainly done in the investigation of the pancreatic function and the diagnosis of diseases of the pancreas. In acute

pancreatitis, serum amylase activity increased within 2 to 12 hours of the onset of the disease with maximal levels in 12-72 hours and the level returns to normal by the third or fourth day.

There is 4 to 6 fold increased in amylase activity above the reference limit. However, up to 20% of the cases may have normal amylase activity in the serum. A significant amount of serum amylase is excreted in the urine, so the rise in serum amylase is reflected in the rise of urine amylase activity.

In quiescent chronic pancreatitis both the serum and urine amylase activity is found to be subnormal.

Amylase activity assay is also used in detecting the development of complications following acute pancreatitis like, pseudocyst, ascites, pleural effusion etc. Pacreatic abscess and traumatic lesion to the pancreas, e.g surgical trauma, may cause transcient rise in serum amylase activity. Pancreatic cancer leading to the obstruction of the duct may elevate the serum amylase activity

Salivary gland

Any lesion to the salivary gland due to infection (mumps), surgical traumaor any other type of trauma, calculus and tumors may lead to high amylase activity in the serum which is mainly of S-type.

Other causes of hyperamylasemia include renal insufficiency, tumor of the lung and ovary, cholecystitis, ruptured ectopic pregnancy, cerebral trauma, diabetic ketoacidosis.