Alanine aminotransferase (ALT) also known as glutamate pyruvate transaminase (GPT) is a transaminase. ALT catalyses the transfer of the aminogroup of L-alanine to a ketoglutarate to give L-glutamate. The highest levels are found in the liver and kidneys, and in smaller amounts in heart and skeletal muscle.

ALT concentration is increased when hepatic cells are damaged (liver cell necrosis or injury of any cause). Indeed, viral and toxic hepatitis induce a market elevation of ALT activity in serum. Intake of alcohol, delirium tremens, and administration of various drug induce slight or moderate elevation of ALT. ALT concentration in serum is also slightly increased in various conditions such as : muscular dystrophy, haemolytic disease, myocar-dial infarction....

ALT is more liver specific than AST (Aspartate aminotrasferase). Measurement of both AST and ALT has some value in distinguishing hepatitis from other parenchymal lesions.

ALT serum level can decrease in case of vitamin B_6 deficiency.

PRINCIPLE :

Kinetic determination of the alanine aminotransferase (ALT) activity :

L-Alanine + □- Ketoglutarate --------> Pyruvate + L-Glutamate

Pyruvate + NADH + H+ --------> L-Lactate + NAD+

REAGENTS :

01. Enzyme Reagent 4x10ml

02. Substrate Reagent 1x10 ml

The reagents are ready to use and usable to the expiration date when stored at 2 - 80C & Protected from light, if contamination is avoided.

SAMPLE :

Serum
Heparin or EDTA plasma

EXPECTED RANGE:

Normal : <40 U/L

LINEARITY :

S G P T kit is linear upto 300 U/L

INSTRUCTIONS :

1. The reagents R1 & R2 contain less than 0.1 % sodium azide.Sodium azide can react with copper and lead plumbing to form explosive metal azides.
2. Use clean or single use glass material only to avoid contaminations.
3. High ALT values may induce falsely low results due to the depletion of the substrate (total consumption of NADH before reading of the result). If an analyser is used, verify the presence of a depletion factor on the application.

DIRECTIONS FOR USE ON ANAL YSERS :

Reaction Type : Kinetic with factor
Wave Length : 340nm
Incubation Temp : 37^0C
Incubation Time : 1 min.
Read Time : 3 min
No. of Readings : 4
Interval Time : 1 min.
Sample Volume : 0.1 ml
Reagent Volume : 1 ml
Unit : U/L
Factor : 1746

PREPARATION AND STABILITY OF WORKING REAGENT:

Mix 4 volumes of the reagent 1 with 1 volume of reagent 2 this working reagent is stable upto 3 weeks at 2-8°C.

PROCEDURE:

One Reagent procedure

Working Reagent : 1ml
Sample : 0.1 ml

Mix and after a 1 minute incubation, measure the change of optical density per minute (□OD/min.) during 3 minutes.

Two Reagent procedure

Reagent 1 : 1ml
Sample : 0.125 ml
Mix, wait 1 minute and add
Reagent 2 : 0.250 ml

Mix and after a 1 minute incubation, measure the change of optical density per minute (□OD/min.) during 3 minutes.

CALCULATION:

At 340nm, with the one-reagent procedure and the two reagent procedure: Activity (U/L)=□OD/min. x 1746.

Determination of serum Amylase

Amylase is an enzyme belonging to the class of hydro lases . It catalyzed the break down of starch and glycogen starch consists of both amylose and amylopectin . Amylose is along of glucose molecules linking by α (1 - 4) glycosidic bonds and amylopectin is linked by α (1 - 6) linkages at the branch points .

Amylase sources in the body :-

1. Pancreas and the Salivary glands are the major tissue sources of serum amylase .
2. Skeletal muscle , small intestine and the fallopian tubes lesser concentrations .

Digestion of starch begins in the mouth with the hydrolytic action of Salivary , Salivary amylase activity pancreatic amylase then preform the major digestive action of starch .

Types of amylase:

1- α-amylase

α-amylases names 1,4-α-D-glucan glucanohydrolase; glycogenase; saccharogen amylase, it is breaks down long-chain carbohydrates ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin.

2- β-Amylase

It names 1,4-α-D-glucan maltohydrolase; glycogenase;. β-amylase catalyzes the hydrolysis of the second α-1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time.

3- γ-Amylase

It names: Glucan 1,4-α-glucosidase; amyloglucosidase; Exo-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase; 1,4-α-D-glucan glucohydrolase). Unlike the other forms of amylase, γ-amylase is most efficient in acidic environments and has an optimum pH of 3.

Amylase properties

1. It active in PH = 6.7 – 7 .
2. It is peaceful at room temperature with one week .
3. It is active at 37 ℃ .
4. Activity increase with Ca2+ and some anions such as Cl - , Br - , NO3- , ClO3- , HPO4= .
5. It is smaller size , the molecular weight of (50 – 55) K.D therefore it is appear in the urine .

Amylase is the serum enzyme most commonly relied up on for detecting pancreatic disease and it is useful in the diagnosis of acute pancreatitis in which significant increases in serum concentrations occure about 75 % of patients .

Clinical Sinificance :-

Amylase levels are increased in serum in many conditions

1. Pancreatic carcinoma .
2. Intestinal infuction .
3. Intestinal obstruction .
4. Pancreatic trauma .
5. Hepatitis .
6. Liver cirrhosis
7. Cholecystitis .
8. Diabetic keto acidosis .
9. Salivary gland lesions .

Principle :-

All methods depend on bondying of serum with substrat for this enzyme (starch) . starch hydrolysis by amylase to produce smaller molecules .

Amylase α-glucosidase
2Glucose Maltose Starch

Normal value :-

160 – 180 Somogyi / dL

Quantitative determination of Acid Phosphatase (ACP)

PRINCIPLE

Hillmann method: acid Phosphatase activity present in the sample is determined according to the modified method of Hillmann. ACP α -naphtyl-phosphate + H2O α- naphtol + Phosphate α- naphtol + Fast Red TR Azo Dye α -naphtol reacts with a diazoted compound forming a colour with a maximum of absorbance at 405 nm. Tartrate is used as specific of the prostatic fraction.

CLINICAL SIGNIFICANCE

Acid phosphatase is an enzyme present in almost all weaves of the organism, being particularly high in prostate, stomach, liver, muscle, spleen, erythrocytes and platelets. High levels of acid phosphatase are found in prostatic phatologies as hypertrophy, prostatitis or carcinoma. In hematological disorders, bones or liver diseases as well as in Paget's or Gaucher's diseases. Decreased serum acid phosphatase has no clinical significance diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PREPARATION

Working reagent (WR): Dissolve one tablet of R 2 Substrate in 2ml of R 1 Buffer. Cap and mix gently to dissolve contents. Stability: 2 days at 2-8°C or 6 hours at room temperature.