Experiment 6

Determination of the enzyme ALT or SGPT activity in serum by enzymatic method using Biophotometer

Background:
Alanine aminotransferase (glutamate pyruvate transaminase) belongs to the group of transaminases, which catalyse the conversion of amino acid to the corresponding α-keto acids via the transfer of amino groups; they also catalyse the reverse process. Although higher activities exist in the liver, minor activity can also be detected in the kidneys, heart, skeletal muscle, pancreas, spleen and lungs. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction. Although, both serum aspartate aminotransferase (AST) and ALT found to be elevated whenever disease processes affect liver cell integrity, ALT is more liver specific enzyme. Moreover, elevations of ALT persist longer than elevations of AST activity.

Clinical Significance:
ALT is a cellular enzyme, found in highest concentration in liver and kidney. High levels are observed in hepatic disease like hepatitis, diseases of muscles and traumatism, its better application is in diagnosis of the disease of the liver.

Principle:
Alanine aminotransferase (glutamate pyruvate transaminase) catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH.

$$\text{Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{Glutamate}$$

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

The rate of decrease in concentration of NADH measured photometrically at 340nm is proportional to the catalytic concentration of ALT present in the sample.

Reagents:

R1 (buffer)	Tris pH 7.8	100mmol/l
	L-Alanine	500mmol/l
	Lactate dehydrogenase (LDH)	1200U/l
R2(substrate)	NADH	0.18mmol/l
	α-ketoglutarate	15mmol/l

Specimen: serum

Specimen Preparation:
Separate serum from cells by centrifugation. ALT activity is stable at 2-8ºC for 7 days.

Reagent preparation:
Working reagent (WR): mix 1volume of R2 with 4volume of R1. Reagent is stable for 21days at 2-8ºC and 72 hours at room temperature.

Working reagent (WR)		
R1	4 volume	
R2	1 volume	

Procedure:

1. Set the spectrophotometer at 340 nm and adjust to zero with dH_2O. This is the blank reading.
2. Prepare a tube as follows,

WR	1.0 ml
Sample	100 µl

3. Mix and incubate for 1 minute.
4. Transfer about 200µl to a cuvette and read the absorbance of the sample. This is the absorbance reading at 0 minute.
5. Start the stopwatch and read the absorbance at 1 minute interval for 3 minutes at 340 nm.

Reading no.	Time (minute)	Absorbance
1	1	
2	2	
3	3	
4	4	

Calculation:
Calculate the difference of absorbance and the average absorbance difference per minute (ΔA/min).

Difference of absorbance (ΔA)		
ΔA1	absorbance at 1min-absorbance at 2 min	
ΔA2	absorbance at 2 min-absorbance at 3 min	
ΔA3	absorbance at 3 min-absorbance at 4 min	

Average Absorbance difference per minute (ΔA/min) = {ΔA1 + ΔA2 +ΔA 3}/3

Reference value (At 37^0C):

Men: up to 40U/L **Women:** up to 32U/L

Experiment 6b

Determination of the enzyme AST or GOT activity in serum by enzymatic method using Biophotometer

Background:

Aspartate aminotransferase (glutamate oxaloacetate transaminase) belongs to the group of transaminases, which catalyze the conversion of amino acids to the corresponding α-keto acids via the transfer of amino groups; they also catalyze the reverse process. AST is commonly found in human tissue. Although heart muscle is found to have the most activity of the enzyme, significant activity has also been seen in the brain, liver, gastric mucosa ,adipose tissue, skeletal muscle, and kidneys. AST is present in both cytoplasm and mitochondria of cells. In cases with mild tissue injury, the predominant form of AST is that from the cytoplasm, with a smaller amount coming from the mitochondria. Severe tissue damage results in more of the mitochondrial enzyme being released. Elevated levels of transaminases are indicative of myocardial infarction, hepatopathies, muscular dystrophy and damage to the internal organs. Increased levels of AST however are generally a result of viral or toxic hepatitis and obstructive jaundice. Following a myocardial infarction, serum levels of AST are elevated and reach a peak 48-60 hours after onset.

Clinical Significance:

AST is a cellular enzyme, found in highest concentration in heart muscle,the cells of the liverthe cells of the skeletal muscle and in smaller amounts in other weaves. An elevated level of AST in the serum is not specific of the hepatic disease,is mainly to diagnostic and to verify the course of this disease with other enzymes like AST. Also it is used to control the patients after myocardial infarction,in skeletal muscle disease etc.

Principle:

Colourimetric methods based on formation of the chromogenic dinitrophenylhydrazone of pyruvate have been in wide use. However the accuracy of these methods is limited. Since dinitrophenylhydrazine reacts with α-ketoglutarate as well as pyruvate, the absorbance value for reagent blank is usually high. The series of reactions involved in the assay system are as follows:

1. The amino group is enzymatically transferred by AST present in the specimen from aspartate to the carbon atom of α-oxoglutarate yielding oxaloacetate and L-glutamate.
2. Oxaloacetate formed is measured in its derivative form, 2, 4 dinitrophenylhydrazone.

DL Aspartate + α-oxoglutarate $\longrightarrow$ Oxaloacetate +L- Glutamate

The intensity of the colour produced is directly proportional to the enzyme activity. It is determined by measuring the increase in absorbance at 530-550nm.

Reagents:

R1 (buffer)	Tris pH 7.8	100mmol/l
	L-Alanine	500mmol/l
	Lactate dehydrogenase (LDH)	1200U/l
R2(substrate)	NADH	0.18mmol/l
	α-ketoglutarate	15mmol/l

Specimen: serum

Specimen Preparation:

Separate serum from cells by centrifugation. AST activity is stable at 2-8ºC for 7 days.

Reagent preparation:

Working reagent (WR): mix 1volume of R2 with 4volume of R1. Reagent is stable for 21days at 2-8ºC and 72 hours at room temperature (15-25ºC).

Working reagent (WR)		
R1	4 volume	
R2	1 volume	

Procedure:

1. Set the spectrophotometer at 340 nm and adjust to zero with dH_2O. This is the blank reading.
2. Prepare a tube as follows,

WR	1.0 ml
Sample	100 µl

3. Mix and incubate for 1 minute.

4. Transfer about 200µl to a cuvette and read the absorbance of the sample. This is the absorbance reading at 0 minute.

5. Start the stopwatch and read the absorbance at 1 minute interval for 3 minutes at 340 nm.

Reading no.	Time (minute)	Absorbance
1	1	
2	2	
3	3	
4	4	

Calculation:
Calculate the difference of absorbance and the average absorbance difference per minute (∆A/min).

Difference of absorbance (∆A)		
∆A1	absorbance at 1min-absorbance at 2 min	
∆A2	absorbance at 2 min-absorbance at 3 min	
∆A3	absorbance at 3 min-absorbance at 4 min	

Average Absorbance difference per minute (∆A/min) = {∆A1 + ∆A2 +∆A 3}/3

Reference value (At 37^0C):

Men: up to 38 U/L **Women:** up to 31 U/L