

AIM: ESTIMATION OF TRANSAMINASES (AST/ALT)

Transaminases are the enzymes that catalyzes transfer of amino group from alpha-amino acid to alpha-oxoacid, thus forming a different alpha-amino acids and alpha-oxoacid.

1. Aspartate aminotransferase (AST), also known as SGOT: has both cytoplasmic and mitochondrial forms. It catalyzes the following reaction:



The enzyme is found in heart, liver, kidney, skeletal muscles.

2. Alanine aminotransferase (ALT), also known as SGPT: cytoplasmic enzyme. It catalyzes the following reaction:



It is found in liver and kidney and in small quantities in heart and skeletal muscles.

Estimation of ALT

Method used:

Colorimetric method

Principle:

ALT catalyzes the reaction between L-alanine and alpha-ketoglutarate forming pyruvate and L-glutamate. The co enzyme used in the reaction is pyridoxal phosphate. To determine the activity of ALT, serum is treated with alanine and alpha-ketoglutarate with added 2,4 dinitrophenyl hydrazine. Thus as pyruvate is formed the colour is changed to reddish brown (hydrazone formation in alkaline medium), the intensity of which is proportional to the activity of enzyme present in the serum.

Procedure:

	T	C	S	B
Buffered solution	1ml	1ml		
Serum	0.2ml			
Standard	-	-	0.2ml	
Incubate in water bath at 37 degrees for 30minutes.				
2,3 DNPH	1ml	1ml	1ml	1ml
Serum		0.2ml		

Shake well and keep at room temperature for 20 minutes

NaOH 10ml 10ml 10ml 10ml

Shake well and keep at room temperature for 10 minutes.

Read absorbance at 520nm by setting zero with blank and note the OD values.

Calculation:

$$\begin{aligned} \text{ALT activity in serum} &= \frac{\text{OD of T} - \text{OD of C}}{\text{OD of S} - \text{OD of B}} \times \text{Concentration of standard} \\ &= \frac{\text{OD of T} - \text{OD of C}}{\text{OD of S} - \text{OD of B}} \times 0.4/0.2 \times 1000/30 \\ &= \frac{\text{OD of T} - \text{OD of C}}{\text{OD of S} - \text{OD of B}} \times 66.66 \text{ IU/l} \end{aligned}$$

Estimation of AST

Method used:

Colorimetric method

Principle:

The transamination reaction between L-aspartate and alpha-oxoglutarate is catalyzed by AST to form L-glutamate and alpha-oxaloacetate. Coenzymes used in this reaction is pyridoxal phosphate. For estimating the AST activity, when serum is treated with aspartate and alpha ketoglutarate in presence of 2,4 dinitrophenylhydrazine, reddish brown hydrazine is formed in alkaline medium. The enzyme activity is proportional to the colour intensity.

Procedure:

	T	C	S	B
Buffered substrate	1ml	1ml		
Serum	0.2ml	0.2ml		
Standard			0.2ml	
Incubate in water bath for 30minutes				
2,3 DNPH	1ml	1ml	1ml	1ml
Shake well and keep at room temperature for 20minutes				
NaOH	10ml	10ml	10ml	10ml

Shake well and keep at room temperature for 10minutes.

Read OD values at 520nm and note them.

Calculations:

$$\begin{aligned} \text{AST activity in serum} &= \frac{\text{OD of T} - \text{OD of C}}{\text{OD of S} - \text{OD of B}} \times \text{concentration of standard} \\ &= \frac{\text{OD of T} - \text{OD of C}}{\text{OD of S} - \text{OD of B}} \times 0.4/0.2 \times 1000/60 \\ &= \frac{\text{OD of T} - \text{OD of C}}{\text{OD of S} - \text{OD of B}} \times 33.33 \text{ IU/L} \end{aligned}$$

INTERPRETATION:

Normal values:

AST = 10-30 U/L

ALT = 10-40 U/L

Other methods:

1. Spectrophotometric method/ enzymatic method
2. Chromatographic methods
3. Isotope methods

Clinical significance:

1. Serum AST rises in heart diseases after 6-8hrs of chest pain.
2. Rise in serum AST is also seen in muscular dystrophy and pulmonary embolism.
3. Serum AST and ALT levels are on higher sides in liver diseases like alcoholic hepatitis, hepatic cirrhosis, liver carcinoma.
The activity of AST and ALT rises to 5-10 times in liver carcinoma cases.
Approx. 20 times in toxic hepatitis
4. ALT/AST ratio, known as De Ritis ratio, is less than 1 in liver diseases mostly except in case of alcohol induced liver disease, where ration is >1.