Cihan University - Sulaimaniya

Clinical Biochemistry

Lab. 7

Measurement of ALT

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Principle of ALT Measurement

Colorimetric method developed by Tonhazy, White, and Umbreit and adapted for the determination of the activity in serum by Reitman and Frankel. Reaction scheme is as follows:

$$\text{L-Alanine} + \text{2-Oxoglutarate} \xrightleftharpoons{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

Then, Pyruvate reacts with 2, 4 DNPH to form 2, 4 Dinitrophenylhydrazones, which absorbance at 505 nm in alkaline solution is proportional to AST or ALT activity in the reactional mixture.

Procedure

1- STANDARD CURVE ESTABLISHMENT:

Pipette into Test tubes (mL):						
Tube number:	1	2	3	4	5	6
Demineralized water	0.200	0.200	0.200	0.200	0.200	0.200
R2 (Substrate)	1	0.900	0.800	0.700	0.600	0.500
R4 (Standard)	--	0.100	0.200	0.300	0.400	0.500
R3 (Dye)	1	1	1	1	1	1
Mix. Let stand for 20 minutes at room temperature. Add:						
NAOH 0.4 N	10	10	10	10	10	10
Mix. Let stand 5 minutes and read absorbances at 505 nm against water.						
TGP (IU/L)	0	40	80	140	225	325

1- ASSAYS:

Pipette into test tubes:	
Reagent R2	1 mL
Incubate for 5 minutes at 37°C. Add:	
Serum	200 µL
Mix and incubate at 37°C during:	Exactly 30 minutes
Reagent R3	1 mL
Mix and let stand 20 minutes at room temperature. Add:	
NaOH 0.4 N	10 mL
Mix. Let stand 5 minutes and read absorbances at 505 nm against water.	

Standard Curve and Calculation

Calculate the result as follows:

- ✓ Refer to enclosed Standard Curves (batch specific)

  or

- ✓ Plot Standard Curves on millimeter paper (Absorbances) handling as indicated in table 1.

  Abscissa: Units (IU/L)

  Ordinate: Absorbances

Transfer “Assay” absorbances on Standard Curve and read activity (IU/L)

What does it mean if ALT result is high?

High levels of ALT in your blood can be due to damage or injury to the cells in your liver. An increased ALT level may indicate the following conditions:

- Alcohol-induced liver injury.
- **Fatty liver disease (too much fat in your liver).**
- **Hepatitis (liver inflammation).**
- **Cirrhosis**
- **Drug toxicity to your liver.**
- **Liver tumor or liver cancer.**
- **Hemochromatosis (having too much iron in your body).**

Reference Interval

EXPECTED VALUES (2)	
ALT (IU/L)	at 37°C
New-born, Infants	13-45
Men	10-40
Women	7-35

Each laboratory should establish its own normal ranges for the population it serves.