[BCH 322]

Method of Enzyme Assay

1- Discontinuous assay (colorimetric assay)

Method

Type of cuvette used ----------

Prepare each tube as follows:

	BLANK	**SAMPLE**
ALT Reagent	0.5 ml	0.5 ml
Pre-warm at 37 °C for 5 minutes and add:		
Distilled Water	0.1 ml	-
Serum Sample	-	0.1 ml
Mix, and incubate at 37 °C for exactly 30 minutes, and add:		
Color Reagent (DNPH)	0.5 ml	0.5 ml
Mix, and return at 37 °C for exactly 10 minutes, then add:		
Color Developer (NaOH)	5.0 ml	5.0 ml
Mix, and return to 37 °C for exactly 5 minutes. Read absorbance of all tubes at 546nm against blank.		

Result

Absorbance at 546 nm = ……

ALT (SGPT) activity of serum sample (from graph)= ……U/L

2- Continuous assay (UV/ kinetics)

Method

Type of cuvette used ----------

Prepare the following:

ALT Reagent	3 ml
Pre-warm at 37°C for 3 minutes and add	
Serum Sample	0.2 ml
Mix and incubated at 37 °C for 1 minute, then read absorbance (at 340 nm against distilled water) every minute for 3 minutes) and determine ΔA/min	

Choose the following on the spectrophotometer:

2) Applications → 2) Simple Kinetics → wave length (340 nm) → 1) Seconds → Duration (180 sec = 3 min) → Intervals (60 sec= 1 min) → Print Data Table (off) → Press start (2 times)

Result

Time	*Absorbance 340nm*		*ΔA/min=((A1-A2)+(A2-A3))/2*
1 min	*A1*		
2 min	*A2*		
3 min	*A3*		

Calculation

ALT Activity (U/L) = ΔA/min x 1768

ALT Activity = ……..U/L