SGPT

(ALANINE AMINOTRANSFERASE)
(DNPH Method)

BIO LAB DIAGNOSTICS
ISO 9001:2008
ISO 13485:2012
CE

CLINICAL SIGNIFICANCE
Increased levels are associated with various liver damages and hepatitis.

PRINCIPLE
Alanine aminotransferase catalyses the following reaction :

L-alanine + oxo-2glutarate —GPT→ pyruvate + L-glutamate

The GPT activity controls the quantity of pyruvate formed. This keto-acid may be assayed by the formation of 2, 4, dinitro phenylhydrazone which yields a brown colour in alkaline medium. The intensity of this colour is a function of the quantity of pyruvate formed and thus the catalytic activity of alanine aminotransferase.

REAGENTS COMPOSITION

1. Buffer - substrate	
Phosphate buffer (pH 7.4)	100 mMol/L
L-alanine	200 mMol/L
Oxo-2-glutarate	2 mMol/L
2. Chromogen	
2.4-Dinitro phenylhydrazine	1 mMol/L
Hydrochloric Acid	1 Mol/L
3. Standard	
Sodium pyruvate	2 mMol/L
4. Sodium Hydroxide	
Sodium hydroxide	4 Mol/L

STORAGE AND STABILITY
When stored tight capped 2-8°C and protected from bright light the reagents are stable until the expiry date stated on each labels. Keep Reagent 4 (Sodium Hydroxide) tight Capped at room temperature. Avoid any bacterial contamination. Reagent 4 when diluted and kept in polyethylene vials is stable for at least 8 days between 18 and 25°C.

SAMPLE
Use fresh serum which has no signs of haemolysis. No prior patient preparation is needed

LIMITATIONS
This method is not linear. Each kit should be quality controlled and calibrated for its activities corresponding to the enzyme. For more accuracy a control must be run along with the test adding serum after Step 4. Any colour shows above the blank of the standard Tube no 1 of calibration graph) must be deducted from the O.D. of the test.

RESULTS
Compare the Optical Density of the Test against the standard calibration graph, any sample having activity greater than 90 Karmen Units/mL must be diluted with 0 9% saline and be confirmed by reassay. (Multiply the result by dilution factor).

PROCEDURE

1. **Dilute reagent 4, 1 mL to 10 mL**
   Reagent 4 ..... 1 volume
   Distilled Water ..... 9 volume

2. **Into a test tube, introduce :**
   Reagent 1 ..... 0.5 mL
   Incubate at 37°C for 5 minutes.

3. **Add : serum ..... 0.1 mL**
   Mix carefully, Leave for exactly 30 minutes at 37°C.

4. **Add : Reagent 2 ..... 0.5 mL**
   Mix well. Let stand 20 minutes at 18-25°C.

5. **Add rapidly whilst mixing :**
   Reagent 4, working ..... 5 mL

6. **After exactly 5 minutes, read the optical density against distilled water at 530 nM (510 - 550 nM Green filter)**

CALIBRATION

1. Into 5 test tubes labelled as under introduce :

	Tube 1 mL	Tube 2 mL	Tube 3 mL	Tube 4 mL	Tube 5 mL
Distilled Water	0.1	0.1	0.1	0.1	0.1
Reagent 1	0.5	0.45	0.4	0.35	0.3
Standard 3	0	0.05	0.1	0.15	0.2
Reagent 2	0.5	0.5	0.5	0.5	0.5
Mix will, allow to stand 20 Minutes at 18-25°C.					
2. Add rapidly whilst mixing : Working Reagent 4.....	5	5	5	5	5
3. After exactly 5 mins, read the optical density against distilled water at 530 nM (510-550 nM) corresponding GPT activites is Karmen Units/mL	0	28	57	97	150

4. Draw the standard curve, expressing GPT activities on the x-axis and optical densities on the y-axis of a graph.

EXPECTED VALUES
Male : 3.8 to 17.0 Karmen Units/mL

Female : 1.3 to 16 Karmen Units/mL

BIBLIGRAPHY
ACKARMANN and TORO Practical Clin Chem. Little Brown and Co Boston

BERGMEYER H.U. Methods Of Enzymatic analysis 2nd Ed. Vol.II (1974) Academic Press N.Y.

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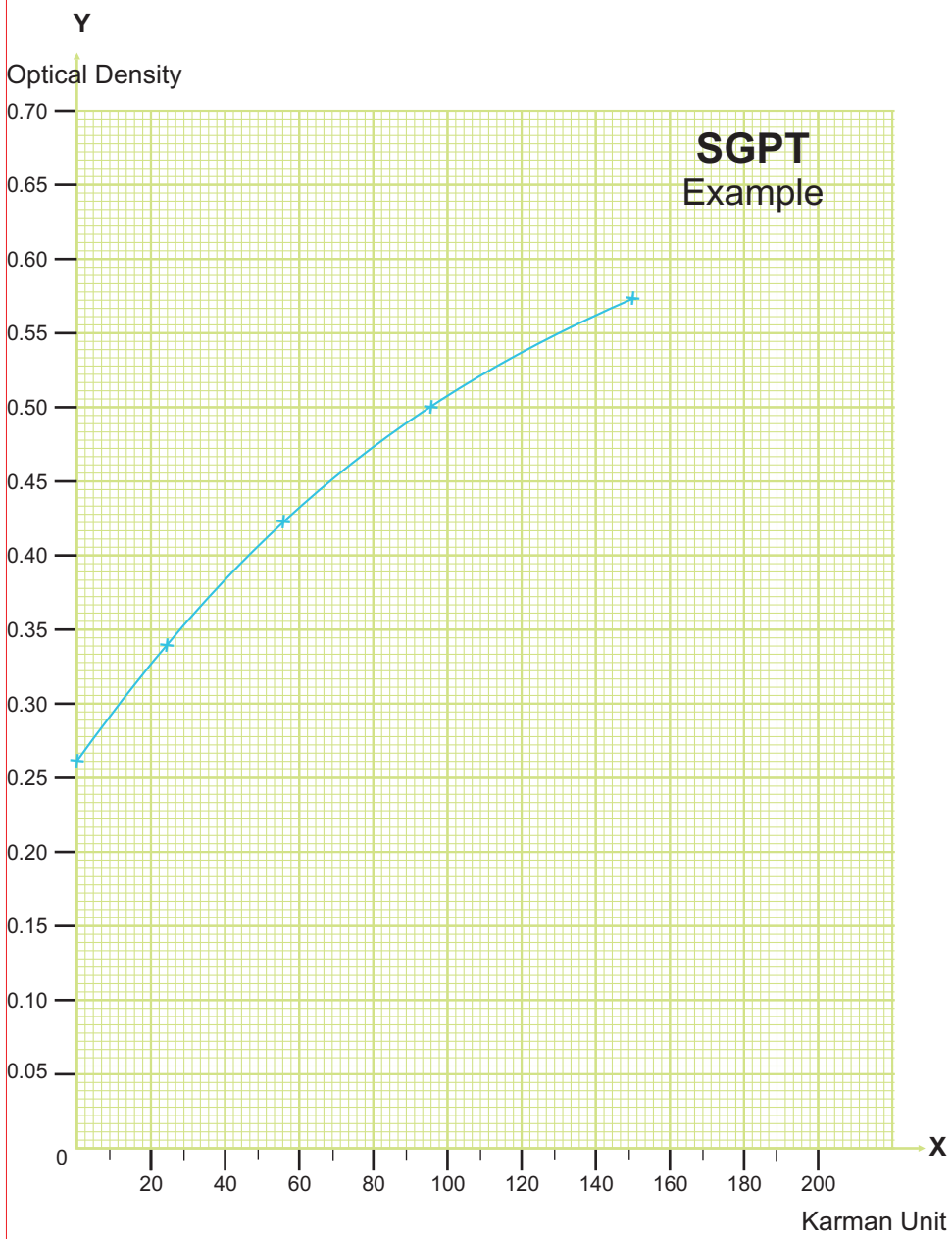
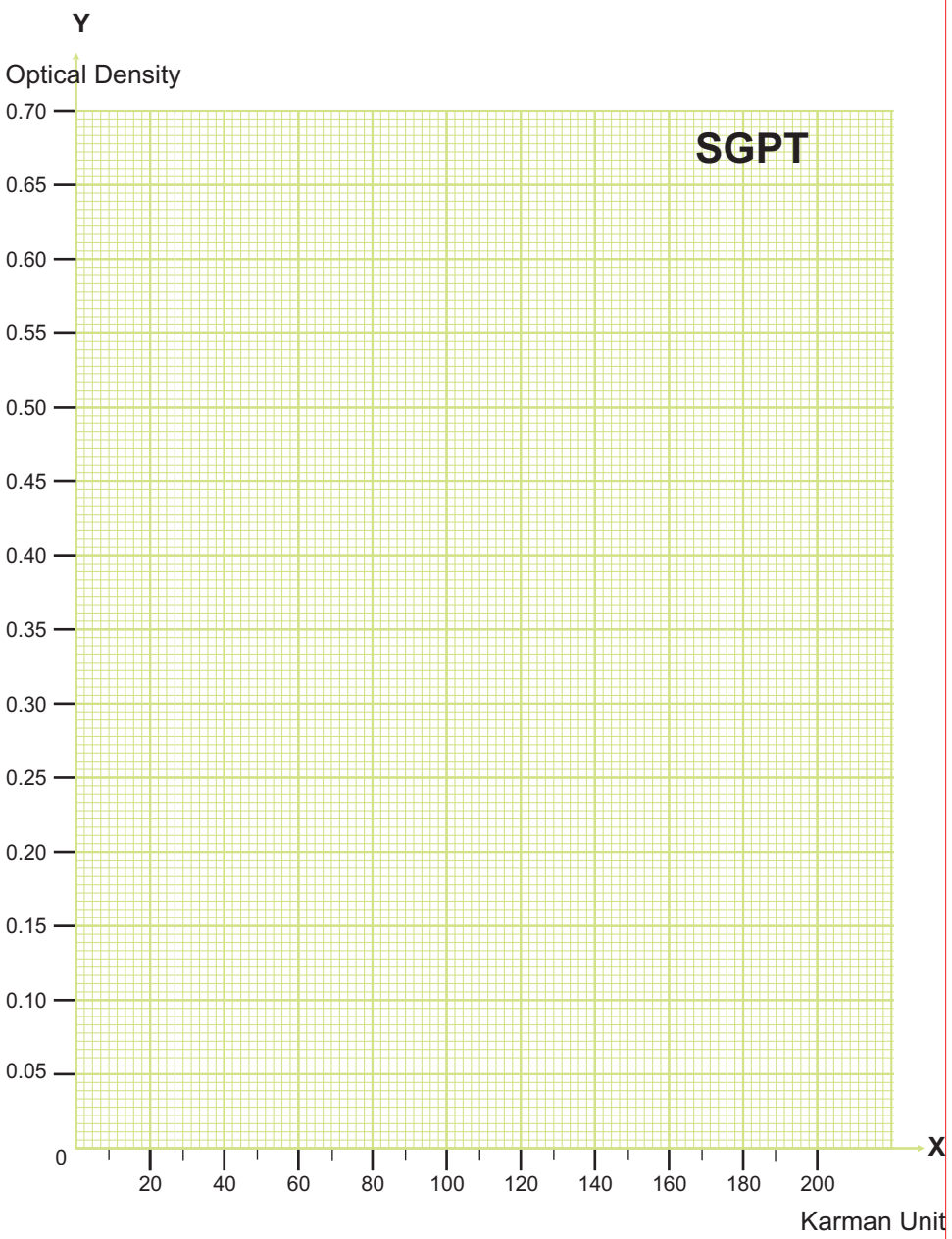

Y
Optical Density
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
SGPT
Example
X
20
40
60
80
100
120
140
160
180
200
Karman Unit


Y
Optical Density
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
SGPT
X
20
40
60
80
100
120
140
160
180
200
Karman Unit