SGOT

(ASPARTATE AMINOTRANSFERASE)
(DNPH Method)

BIO LAB DIAGNOSTICS
ISO 9001:2015
ISO 13485:2016
CE

CLINICALSIGNIFICANCE
A high activity of AsAT is the Clinical indication of myocardial infarctions, noticeable after a few hours of onset and **lasting for 4-5 days.** High levels are also found in case of liver cell damage, muscular dystrophy and dermatomyositis.

PRINCIPLE
Aspartate aminotransferase catalyses the following reaction:

L-aspartate + oxo-2-glutarate —GOT→ oxaloacetate + L-glutamate

GOT activity is shown by the quantity of oxalacetic formed. This ketoacid 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 oxalocacetate formed, and thus the catalytic activity of aspartate aminotransferase.

REAGENTS COMPOSITION

1. Buffer-substrate

Phosphate buffer (pH7.4)	100 mMol/L
L-aspartate	100 mMol/L
Oxo-2-glutarate	2 mMol/L

2. Chromogen

2,4-Dinitro phenylhydrazine	1 mMol/L
HydrochloricAcid	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 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 test reading.

RESULTS
Compare the Optical Density of the Test against the standard calibration graph, any sample having activity greater than 100 Karmen Units/mL must be diluted with 0.9% saline and be

PROCEDURE

	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 60 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 530Nm (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 : Reagent 4, working	5	5	5	5	5
3. After exactly 5 mins, read the optical density against distilled water at 530 nM (510-550 nM) corresponding GOT activites is Karmen Units/mL	0	24	61	144	190

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

EXPECTED VALUES
Male : 4.2 to 28.9 Karmen Units/mL

Female : 2.6 to 27.8 Karmen Units/mL

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

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

BIOLAB DIAGNOSTICS (I) PVT. LTD.
J-245, MIDC, TARAPUR, BOISAR - 401 501. MAHARASHTRA, INDIA.
E-mail: biolab@vsnl.com / www.biolabdiagnostics.com
Customer Care : (+9122) 28088243