(DNPH METHOD)



Reagent Kit is for quantitative estimation of glutamate Oxaloacetate Transaminase activity in serum or plasma.

## BACKGROUND & SYNOPSIS:

Glutamate oxaloacetate transaminase (aspartate amino transferase, AST) is one of the two transaminases used for diagnostic purpose. Transaminase or aminotransferrase carry out a reaction between an amino acid and keto acid, converting amino acid to keto acid and keto acid to amino acid by transferring the amino group to a keto acid. Heart has the highest concentration of GOT, followed by liver, skeletal muscle and kidneys. Estimation of GOT is useful in the diagnosis of myocardial infarction.

The DNPH method was first used by Tonhazey and Cabaud et.al. it was modified by Reitman and Frankel.

SGOT is formulated on the methodology as modified by Reitman and Frankel.

#### PRINCIPLE:

For estimation of GOT sample is allowed to react with alphaketoglutarate and L-Aspartate. Oxaloacetate thus formed reacts with 2,4-dinitrophenyl hydrazine (2, 4-DNPH) which gives a corresponding hydrazone, a brown/red colored complex, in an alkaline medium. This is measured photometrically at 505 nm (490-550 nm or with Green filter).

L-Glutamate Alpha-Ketoglutarate GOT L-Aspartate Oxaloacetate.

## DIAGNOSTIC SIGNIFICANCE:

Glutamate oxaloacetate transaminase is localized in a mitochondria and cytoplasm of normal muscle cells. When cells are damaged or cellular nutrition/blood supply disturbed, the permeability of the cell membrane increases and transaminases are released into the blood stream. An increased serum transaminase level indicates cellular death. Estimation of GOT level is particularly significant in the diagnosis of myocardial infarction, which increases after 3 to 8 hours of the onset of attack and comes back to normal in 4 to 6 days. The duration and extent of increase in levels is proportional to the severity of attack.

# SPECIMEN COLLECTION:

Fresh, fasting, unhemolysed, clear, serum is the specimen of choice. However, plasma collected with heparin, oxalate or citrate as anticoagulants may be used.

## PRECAUTIONS:

SGOT is for Invitro diagnostic use only. Do not use hemolysed samples as GOT content in re blood cells is very high.

## PRESENTATION:

1 SGOT (Substrate) Ready for use. 2 SGOT (Color reagent) Ready for use. 3 SGOT (Alkali Reagent) Dilute 1 + 9 ml PYRUVATE STANDARD 160 U/L (Store at 2-8 °C)

- It is suggested to plot a calibration graph to interpret test results.
- Procedure with graph plotting available on request

# WORKING REAGENT PREPARATION:

Dilute the 3 SGOT in the proportion 1:10 (1 ml of 3 SGOT + 9 ml of distilled water)

## REAGENT STORAGE AND STABILITY:

SGOT reagents are stable until expiry date stated on the label when stored at 2-8 ° C

#### TEST PROCEDURE:

Pipette into Test Tubes	Reagent Blank	Calibrator	Test	
1 SGOT	0.25 ml	0.25 ml	0.25 ml	
Sample			0.05 ml	
Calibrator / Std. 160 U/L	058	0.05 ml		
Mix and incu	ibate at 37°	C for 60 minu	les.	
2 SGOT	0.25 ml	0.25 ml	0.25 ml	
Mix well and	incubate for	20 minutes 3	7°C.	
Sample		\ <del>\</del>	(5.0)	
3 SGOT (Diluted)	1.5 ml	1.5 ml	1.5 ml 1.5 ml	

Mix and read absorbance of all tubes against distilled water at 505 nm or with Green filter.

## CALCULATION:

SGOT (AST) Activity in U/L.

\_x Conc. of standard =(Abs. Test - Abs.Reagent Blank) (Abs. Calibrator - Abs.Reagent Blank) (160 U/L)

NORMAL VALUES: 5-50 UNITS/L

## PROCEDURE LIMIT:

The method is readable up to 300 U/liter. for GOT activity higher than 300 U/liter, dilute it suitably with 0.9% saline and repeat the assay. Apply proper dilution factor to obtain test results.

## **BIBLIOGRAPHY:**

- REITMAN. S. AND FRANKEL, S., America J. of Clinical Pathology, 28; 56 (1957)
- TIETZ. N., Fundamentals of Clinical Chemistry. W.B. Saunders Co., U.S.A., (1970), P 447.

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	IVD	For in vitro diagnostic use only	REF	Catalog #
	2°C 1 8°C	Store between 2-8°C	LOT	Lot Number
	8	Do not use if package is damaged	M	Date of Manufacturing
	<b>W</b>	Manufacturer	2	Use by