Lifechem™ SGOT (AST)-DB

(MODIFIED IFCC UV - KINETIC METHOD)

CLINICAL SIGNIFICANCE:

Serum Glutamate Oxaloacetate Transaminase (SGOT), also called Aspartate Aminotransferase (AST), belongs to the Transferase class of enzymes. This enzyme shows high levels of activity in the heart, liver, skeletal muscles and kidneys. Since its level is seen to increase enormously following Myocardial Infarction (MI), it can be used as supporting evidence in the diagnosis of MI (especially 20-36 hrs after MI). Elevated levels are also seen in Viral/toxic Hepatitis, Hepatic and Cardiac necrosis, Muscular dystrophy and Pulmonary embolism.

Comparison to the colorimetric, End Point Method of Reitman & Frankel, the Modified IFCC Method is superior in terms of its linearity, specificity, reproducibility and rapidity.

TEST PRINCIPLE:

SGOT catalyses the transfer of an amino group from L-Aspartate to α-ketoglutarate. The rate of the reaction is monitored using a coupling enzyme, Malate Dehydrogenase (MDH), whereby the Oxaloacetate formed is converted to Malate in the presence of NADH. The oxidation of NADH is measured by monitoring the decrease in absorbance at 340 nm

$$\text{L-Aspartate} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{SGOT}} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{MDH}} \text{Malate} + \text{NAD}^+$$

NORMAL RANGE:

Male : Up to 37 IU/L at 37^{0}C

Female : Up to 30 IU/L at 37^{0}C

The expected value should be used as a reference only and it is recommended that each laboratory should establish its own normal range.

KIT CONTENTS:

	Code No. KOT1 (5x10ml)	**Code No.** KOT2 (5x20ml)
Reagent 1 Enzyme reagent	5 x 10 ml	5 x 20 ml
Reagent 2 Buffer solution	50 ml	100 ml

SPECIMEN:

a. Unhemolysed Serum / EDTA Plasma.

b. Blood samples may be collected any time, although morning samples are preferred.

c. Samples are stable for a week at 2-8^{0}C and for one month at –10^{0}C. Samples should be brought to room temperature prior to use.

WORKING REAGENT PREPARATION:

For 5x10 ml pack size:

Reconstitute one vial of Enzyme Reagent (1) with 10 ml of Buffer Solution (2).

For 5x20 ml pack size:

- Reconstitute one vial of Enzyme Reagent (1) first with 10 ml of Buffer Solution (2)
- Mix gently to allow dissolution.
- Finally add 10 ml of Buffer solution (2) to make up the volume to 20 ml.

The working Reagent is stable for at least 30 days at 2-8^{0}C.

PROCEDURE:

Pipette into test tube as follows:

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Enzyme reagent	1.0 ml
Serum/ Plasma	0.1 ml

Assay temperature 37^{0}C

Mix well and read absorbance against distilled water at 340 nm as follows:

A_0 -exactly after 1 minute

A_1 A_2, A_3 -exactly after every 30 seconds for 1 minute 30 seconds

Calculate the average change in absorbance per minute (ΔA/min) x1746 x tf

CALCULATIONS:

At 340 nm in IU/L = ΔA/min x1746 x tf

At 334 nm in IU/L = ΔA/min x1780 x tf

At 365 nm in IU/L = ΔA/min x3235 x tf

Temperature conversion factors (tf)

Assay temperature	**Temperature Conversion Factor**
25^{0}C	2.08
30^{0}C	1.54
37^{0}C	1.00

QUALITY CONTROL:

It is recommended to include Assayed Quality control serum (Level I & II) with each assay batch to verify the performance of the procedure. Failure to obtain the proper range of values in the assay of control sera may indicate reagent deterioration,instrument malfunction or procedural errors.

SYSTEM PARAMETERS:

Reaction type (Mode)	: Kinetic	Wave length	: 340 nm
Flow Cell Temp.	: 37^{0}C	Sample volume	: 100µL
Reagent volume	: 1000µL	Factor	: 1746
Delay Time	: 60 Sec	Kinetic interval	: 30 Sec
No. of Readings	: 4	Units	: IU/L
Low normal	: 1	High normal	: 37
Blank	: D.Water	Linearity	: 450
Abs.Minima	: 1.0	Abs. Maxima	: ——
Reaction direction	: Decreasing		

NOTES:

1) Do not leave working reagent at Room Temperature.
2) If ΔA/min exceeds 0.26, repeat the test using serum diluted 1:10 with normal saline. Multiply the result with the factor, 10.
3) Discard the Working Reagent if it shows an initial absorbance below 1.0 against distilled water at 340 nm.
4) Highly icteric and Lipemic samples have to be diluted with normal saline and the result multiplied with the appropriate dilution factor.
5) Hemolysed samples will interfere with the test.
6) Programmes for specific analysers are available on request.
7) For accuracy of results the procedure has to be meticulously followed.
8) As with all diagnostic procedures, the physician should evaluate data obtained by the use of kit in light of other clinical information.

LINEARITY: 450 IU/L

BIBLIOGRAPHY:

1) Begmeryer, H.U. et.al., (1986); J.Clin. Chem. Biochem. 24, 497.
2) IFCC Expert panel on enzymes ,Clin.Chem.Acta; (1976); 70,19.

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