Asritha
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SGPT (MR)

(Modified IFCC method)
(Ready-to-Use)

CLINICAL SIGNIFICANCE

SGPT is found in a variety of tissues but is mainly found in the liver

INCREASES:

Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic diseases. Slight elevation of the enzymes is also seen in myocardial infarction.

METHODOLOGY: Modified IFCC method

PRINCIPLE

SGPT (ALT) catalyzes the transfer of amino group between L-Alanine and a-Ketoglutarate to form Pyruvate and Glutamate. The Pyruvate formed reacts with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT (ALT) activity in the sample.

$$\text{L-Alanine} + \text{a-Ketoglutarate} \xrightarrow{\text{SGPT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\text{LDH}} \text{Lactate} + NAD^{+}$$

REAGENT COMPOSITION:

Tris Buffer 25 mmol/L
L-Alanine - 200 mmol/L
NADH - 0.15 mmol/L
LDH- 2000 U/L
EDTA- 5.0 mmol/L
-Ketoglutarate - 12 mmol/L

LINEARITY:450 IU/L

ASSAY FACTOR : 1746

STORAGE / STABILITY

Contents are stable at 2-8^{0}C till the expiry mentioned on the labels.

REAGENT PREPARATION

Reagents are ready to use.

Working reagent: Reagent is ready for use.

SAMPLE MATERIAL:

Serum, Free from hemolysis. SGPT (ALT) is report to be stable in serum for 3 days at 2-8^{0}C.

ASSAY PROCEDURE

Wavelength / Filter : 340 nm / filter
Temperature : 37°C/30°C/25°C
Light path : 1 cm

SAMPLE START ASSAY:

Pipette into a clean dry test tube labeled as Test (T):

Addition Sequence	Test (T) 37^{0}C
SGPT Reagent	1.0 ml
Incubate at the assay temperature for 1 min. and add	
Sample	0.1 ml

Mix well and read the initial absorbance A_0 after 1 min. and repeat the absorbance reading after 1, 2 & 3 minutes. Calculate the mean absorbance change per min. (A/min).

CALCULATIONS

SGPT (ALT) Activity in U/L 37^{0}C = /A min. x 1746.

TEMPERATURE CONVERSION FACTORS

Assay	Desired Reporting Temperature 370C
25^{0}C	1.82
30^{0}C	1.38
37^{0}C	1.00

LINEARITY

The procedure is linear upto 450 IU/L at 37^{0}C, if the absorbance change (/A min.) exceeds 0.250, use only the value of the first two minutes to calculate the result, or dilute the sample 1+9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 10).

NOTE:

Samples having a very high activity show a very low initial absorbance as most of the NADH is consumed prior to the start of measurement. If this is suspected then dilute the sample and repeat the assay.
The working reagent or the combined reagent should have an absorbance above 1.0 against distilled water at 340 nm. Discard the reagent if the absorbance is below 1.0

QUALITY CONTROL

To ensure adequate quality control each run should include assayed Normal and Abnormal controls.

NORMAL REFERENCE VALUES

Serum (Males) : upto 40 U/L at 37^{0}C
Serum (Females) : upto 31 U/L at 37^{0}C
It is recommended that each laboratory establish its own normal range representing its patient population.

REFERENCES:

IFCC methods for the measurements of catalytic concentrations of enzymes, J.Clin. Chem. Clin. Biochem. (1986) 24:481

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