SGPT (IFCC without PDP.)

INTENDED USE

Bioline SGPT (ALT) is used for the quantitative determination of alanine amino transferase in human serum.

CLINICAL SIGNIFICANCE

The enzyme alanine amino transferase is widely reported in a variety of tissue sources. The major source of ALT is of hepatic origin and has led to the application of ALT determinations in the study of hepatic diseases. Elevated serum levels are found in hepatitis, cirrhosis, and obstructive jaundice. Levels of ALT are only slightly elevated in patients following a myocardial infarction. UV methods for ALT determination were first developed by Wroble wskiand La Due in 1956. The method was based on the oxidation of NADH by lactate dehydrogenase (LDH).In 1980, the International Federation of Clinical Chemistry recommended a reference procedure for the measurements of ALT based on the Wroble wski and LaDue procedure.

METHOD AND PRINCIPLE

The SGPT (ALT) reagent conforms to the formulation recommended by the IFCC. The enzymatic reaction sequence employed in the assay of ALT is as follows:

$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\text{LDH}} \text{Lactate} + NAD^{+} + H_2O$$

The pyruvate formed in the first reaction is reduced to lactate in the presence of lactate dehydrogenase and NADH. The activity of ALT is determined by measuring the rate of oxidation of NADH at 340 nm. Endogenous sample pyruvate is converted to lactate by LDH during the lag phase prior to measurement.

REAGENT COMPOSITION

Buffer	100mmol/L
L-Alanine	400 mmol/L
LDH	800 U/L
α- Ketoglutarate	13mmol/L
NADH	0.2mmol/L

Non-reactive stabilizers and preservatives

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.
   CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact. Specimens should be considered infectious and handled appropriately.
2. Use distilled or deionized water where indicated.

WORKING REAGENT PREPARATION

The working reagent is prepared by mixing 4 volumes of R1 with one 1 volume of R2 in a disposable container.
Example: 0.8 ml R1 + 0.2ml R2.

STORAGE AND STABILITY

1. Reagents are stable until the expiration date on their respective labels, when properly stored at 2 - 8°C and protected from light. Reagents should appear clear and colorless. The working reagent is stable for 4 weeks at 2 - 8°C.

REAGENT DETERIORATION

The reagent should be discarded if:

1. Turbidity has occurred; turbidity may be a sign of contamination.
2. The reagent fails to meet linearity claims or fails to re-cover control values in the stated range.
3. The reconstituted reagent has a reagent blank absorbance less than 0.800 at 340 nm.

SPECIMEN COLLECTION AND STABILITY

Non-hemolyzed serum is the specimen of choice, yet EDTA treated plasma or lithium heparinized plasma can be used. Whenever possible, specimens should be separated and analyzed on the day of collection. Store serum in stoppered tubes. The enzyme in serum is reportedly stable for a minimum of 7 days at 2-8°C and 3 months at -20 deg c.

INTERFERENCE

Pyridoxal phosphate can elevate ALT values by activating the apo enzyme form of the transaminase. Pyridoxal phosphate may be found in water contaminated with microbial growth.
High levels of serum pyruvate may also interfere with assay - performance. Young, et al., has given a comprehensive list of drugs and other substances that may interfere with the ALT estimation .

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.

Wavelength : 340nm
Temperature : 37°C

	Test
ReagentR1	800µL
ReagentR2	200µL
Sample	50µL

Mix and aspirate in the analyzer ,after 1 minute of delay measure the change of optical density per minute (ΔOD/min.) during next 2 minutes.

High Linearity Procedure

For Samples above 800 IU/L record the change in OD per min for 60 Sec only, after a delay of 30 sec and multiply with the factor. With this procedure linearity of 1600 IU/L can be achieved

CALCULATION

SGPT (ALT) IU/L= ΔA/Min X 3336

LIMITATIONS

Using normal manual procedure reagent is linear upto 800 IU/L. For Sample that have ALT values greater than 800 IU/L follow the High Linearity Procedure.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established ALT values may be routinely used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedure errors.

EXPECTED VALUES

Up to 56 IU/L (37°C)

It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments , laboratories and local populations

PERFORMANCE CHARACTERISTICS

1. Linearity: 800 IU/L. For samples above the linearity range dilute and re-run and multiply the obtained result with dilution factor to get correct result.
2. Comparison: Studies between the present method and a similar method yield a correlation of 0.99 and a regression equation
   Y=0.98X+1.32.
3. Precision : Within Run

Mean (IU/L)	S.D.	C.V.%
41.7	0.9	2.2
115	3.3	2.9

Precision: Between Run

Mean (IU/L)	S.D.	C.V.%
42.4	1.5	3.1
118	3.8	3.3

GENERAL TECHNICAL PARAMETERS

Mode	Kinetic
Wavelength (Filter)	340nm
Reaction Direction	Decreasing
Sample Vol.	50µl
Reagent Vol.	1000 µl
Delay Time / Lag Time	60 Sec
Interval Time	60 Sec
No. of reading	2
Measuring time	120Sec
Reagent Blank Abs.(Max)	NLT 0.800
Calibration Method	Fix factor
Factor	3336
Linearity	800 IU/L
High Linearity	1600 IU/L
Decimal Places	1
Temp.	37°C
Unit	IU/L
Ref. Low (Male / Female)	7 IU/L
Ref. High (Male / Female)	56 IU/L

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