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ALT (SGPT) REAGENT SET
(UV-KINETIC METHOD)

ALANINE AMINOTRANSFERASE (ALT) REAGENT SET
For the quantitative determination of alanine aminotransferase in serum.

INTRODUCTION
The enzyme alanine aminotransferase 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 to 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. [1]

UV methods for ALT determination were first developed by Wroblewski and LaDue in 1956.[2] 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 Wroblewski and LaDue procedure.[3] The ALT reagent conforms to the formulation recommended by the IFCC.

PRINCIPLE
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
When reconstituted as directed, the reagent for ALT contains the following:
(Concentrations refer to reconstituted reagent): α-Ketoglutarate 13 mM, L-Alanine 400 mM, NADH 0.2 mM, LDH 800 U/L, Buffer l00 mM, pH 7.5, 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.
2. Specimens should be considered infectious and handled appropriately.
3. Use distilled or deionized water where indicated.

STORAGE AND STABILITY
1. Store dry reagent at 2 - 8°C (refrigerated).
2. The reconstituted reagent is stable for thirty (30) days if immediately refrigerated and for twenty-four (24) hours at room temperature.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity may be a sign of contamination.
2. Moisture has penetrated the vial and caking has occurred.
3. The reagent fails to meet linearity claims or fails to recover control values in the stated range.
4. The reconstituted reagent has a reagent blank absorbance less than 0.8 at 340 nm.

SPECIMEN COLLECTION
This assay is intended for use with serum. Reports indicate that ALT in serum remains stable at 4 - 8°C for a minimum of seven (7) days.[4] Hemolyzed specimens should not be used as erythrocytes contain seven times the ALT activity of serum.

INTERFERING SUBSTANCES
Pyridoxal phosphate can elevate ALT values by activating the apoenzyme form of the transaminase.[5] 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., give a list of drugs and other substances that interfere with the determination of ALT activity.[6]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipetting devices.
2. Test tubes/rack.
3. Timer.
4. Spectrophotometer with capability to read at 340 nm (UV).
5. Heating bath/block (37°C).

GENERAL INSTRUCTIONS
The reagent for ALT is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

PROCEDURE (AUTOMATED)
Consult the appropriate instrument application guide available from us.

PROCEDURE (MANUAL)
1. Reconstitute reagent according to instructions.
2. Pipette l.0 ml of reagent into a 1cm cuvette and allow to equilibrate to 37°C. If the spectrophotometer being used requires a final volume greater than l.0 ml for accurate readings refer to ALTERNATE PROCEDURE.
3. Add 0.10 ml (100µl) of specimen to reagent and mix gently.
4. Maintain solution at 37°C. After 60 seconds, measure the absorbance at 340 nm.
5. Take two additional absorbance readings at one (1) minute interval. Calculate the mean absorbance change per minute (Δ A/min).
6. Multiply the ΔA/min. by 1768 to calculate IU/L of ALT activity.
7. Sample with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two (2).

NOTE:
If the spectrophotometer being used is equipped with a temperature controlled cuvette, the reaction mixture may be left in the cuvette while the absorbance readings are taken.

PROCEDURE NOTES (MANUAL)
Turbid or highly icteric samples may give readings whose initial absorbance exceeds the capabilities of the spectrophotometer. More accurate results may be obtained by using 0.05 ml (50µl) sample and multiplying the final answer by two (2).

CALCULATIONS
One International Unit (IU) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

$$\text{ALT (IU/L)} = \frac{\Delta A/\text{min.} \times TV \times 1000}{\in \times SV \times LP}$$

$$= \frac{\Delta A/\text{min.} \times 1.10 \times 1000}{6.22 \times 0.1 \times 1.0} = \Delta A/\text{min.} \times 1768.$$

(Calculations continued)

Where	ΔA/min.	=	Average absorbance change per minute
	TV	=	Total reaction volume (ml)
	1000	=	Conversion of IU/ml to IU/L
	∈	=	Millimolar absorptivity of NADH
	SV	=	Sample volume (ml)
	LP =		Light path in cm

Example:	1.480	=	Initial absorbance
	1.350	=	absorbance after one (1) minute.
	ΔA/min.	=	1.480-1.360 = 0.12. Then 0.12 x 1768 = 21.2 IU/L

SI UNITS: To convert to SI Units (nkat/L) multiply IU/L by 16.67.

NOTE: If any of the test parameters are altered a new factor must be calculated using the above formula.

ALTERNATE PROCEDURE
1. Reconstitute reagent according to instructions.
2. Pipette 2.8 ml of reagent into a 1 cm cuvette and allow to equilibrate to 37°C.
3. Add 0.2 ml (200 µl) of specimen to reagent, mix gently.
4. Maintain solution at 37°C. after one (1) minute, measure the absorbance at 340 nm. (A_1)
5. After exactly three (3) minutes, read and record absorbance A_2.
6. The difference in absorbance between readings ($A_1 - A_2$) multiplied by the factor of 803.

ALTERNATE PROCEDURE CALCULATIONS

$$\text{ALT (IU/L)} = \frac{(A_1 - A_2) \times 3.0 \times 1000}{3 \times 1 \times 6.22 \times 0.2} = (A_1 - A_2) \times 803$$

$(A_1 - A_2)$ = absorbance change.
Example: If $A_1 = 1.45$, $A_2 = 1.35$
then (1.45-1.35) x 803 = 0.10 × 803 = 80.3 IU/L.

NOTE: If any the above test parameters have been altered, a new factor must be calculated using the above formula.

PROCEDURAL LIMITATIONS
The reagent is linear to 500 IU/L. Sample that have ALT values greater than 500 IU/L should be diluted 1:1 with saline, reassayed and the results multiplied by two (2).

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 either reagent deterioration, instrument malfunction, or procedure errors.

TEMPERATURE CORRECTION [7]
1. If the assay is performed at 37°C but is to be reported at 30°C, multiply the results by 0.7.
2. If the assay is performed at 30°C but is to be reported at 37°C, multiply the results by l.43.

EXPECTED VALUES[8]
Up to 26 IU/L (30°C) Up to 38 IU/L (37°C)
It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS
1. Linearity: 500 IU/L
2. Comparison: Studies between the present method and a similar method yield a correlation coefficient of 0.99 and a regression equation Y = 0.98X + 1.32.
3. Precision studies

Within Run

Mean (IU/L)	S.D.	C.V.%
23.6	1.8	7.9
82.6	2.1	2.5

Run to Run

Mean (IU/L)	S.D.	C.V.%
23.4	1.9	8.0
82.4	1.9	2.3

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