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ALT (SGPT) LIQUID REAGENT (KINETIC METHOD)

Catalog Number: BQ 004D - CR

INTENDED USE

For the quantitative determination of alanine aminotransferase in serum used in routine examination and monitoring of therapy and relapses.

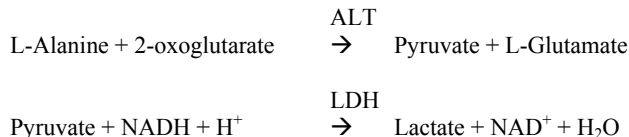
INTRODUCTION

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

Wroblewski and LaDue first developed UV methods for ALT determination 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:



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.

REAGENTS

ALT Liquid Reagents 1 and 2 come in separate containers, and both reagents are clear, colorless liquid in ready to use format. After combining ALT Liquid R1 (Buffer Reagent) and ALT Liquid R2 (Co-Enzyme) the working reagent contains:

L-Alanine	500 mmol/L
LDH	> 1200 U/L
Tris Buffer, pH 7.5	100 mmol/L
2 – Oxoglutarate	15 mmol/L
NADH (Disodium salt)	0.18 mmol/L
Stabilizers and Preservatives	

WARNINGS AND PRECAUTIONS

Normal precautions exercised in handling laboratory reagents should be followed. The reagents contain sodium azide, which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard, or safety information.

REAGENT PREPARATION

The working reagent is prepared by mixing five (5) volumes of R1 with one (1) volume of R2 in a disposable container.

Example: 25 ml R1 + 5 ml R2

REAGENT STORAGE

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.

REAGENT DETERIORATION

1. Discard if either appears cloudy or contains particulate matter.
2. The working reagent is stable for 2 weeks at 2-8°C. The working reagent should be discarded if the initial absorbance, read against distilled water at 340 nm, is below 1.000.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Spectrophotometer capable of absorbance reading at 340 nm and 1 cm light path
2. Constant temperature block or bath, 37 °C, or temperature controlled cuvette well
3. Accurate pipetting devices
4. Test tubes
5. Interval timer

SPECIMEN COLLECTION AND STORAGE

Non-hemolyzed serum is the specimen of choice. Whenever possible specimens should be separated and analyzed on the day of collection. Store serum in stoppered tubes. About 10% ALT is lost 3 days at 4°C and in 1 day at 25 °C.⁵

INTERFERING SUBSTANCES

Hemolysis must be avoided as the concentration of ALT in red cells is roughly 5 times that of serum.² Bilirubin levels up to 40 mg/dL and triglyceride levels up to 2000 mg/dL show no interference in this test. Certain drugs and other substances are also known to affect ALT values.⁵

MANUAL PROCEDURE

1. Prepare ALT working reagent according to instructions.
2. Pipette 1.0 mL of working reagent into tubes labeled “controls”, “patient(s)”, etc.
3. Pre-incubate all tubes at 37°C for at least five minutes.
4. Zero spectrophotometer at 340 nm with distilled water.
5. Add 100 µL (0.10 mL) serum to its respective tube, mix gently and turn to a thermo cuvette.
6. Read and record absorbance at 1 minute. Continue incubating at 37 °C and record absorbance again at 2 and 3 minutes. Rate should be constant.
7. Determine the average absorbance per minute ($\Delta A/\text{min}$), multiply by factor 1768 for results in U/L.
8. Repeat the procedure for each sample.

NOTE: If cuvette is not temperature controlled, incubate samples at 37 °C between readings.

AUTOMATED PROCEDURE

Refer to appropriate application manual available.

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.

CALIBRATION

ALT activity is based on the "micromolar extinction coefficient" of NADH at 340 nm (see "Results" section). The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer. Assaying the ALT contents of a control serum with known ALT values can be used to assure instrument calibration has been performed correctly.

RESULTS

Values are derived based on the "absorptivity micromolar extinction coefficient" of NADH at 340 nm (0.00622). Units per liter (U/L) of ALT/GPT activity is that amount of enzyme, which oxidizes one µmol/L of NADH per minute.

$$U/L = \frac{\Delta A/Min}{Absorbivity} \times \frac{Total Volume}{Sample Volume}$$

$$U/L = \frac{\Delta A/Min}{0.00622} \times \frac{1.100}{0.100}$$

$$U/L = \Delta A/Min \times 1768$$

LIMITATIONS

If the ΔA/min. is greater than 0.342, dilute 1 part sample with 9 parts isotonic saline and re-assay. Multiply the result by 10. ALT values for neonatal patients have not been established with this procedure. Grossly icteric or turbid specimen may require the use of a sample blank.

EXPECTED VALUES

Normal Range: 3 - 35 U/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. Comparison: A group of 128 sera ranging in ALT activity from 7-625 U/L was assayed by the described ALT method and by a similar commercially available ALT reagent. Comparison of the results yielded a correlation coefficient of 0.999 and the regression equation was $y = 0.960x + 3.2$. (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T.)

2. Precision:

	<i>Within-Run</i>	
	<u>Serum 1</u>	<u>Serum 2</u>
Mean ALT (U/L)	25.1	116.0
Std. Deviation (U/L)	0.82	0.90
C.V. (%)	3.25	0.77

	<i>Total Precision</i>	
	<u>Serum 1</u>	<u>Serum 2</u>
Mean ALT (U/L)	25.8	114.8
Std. Deviation (U/L)	1.13	0.8
C.V. (%)	4.40	0.69

Precision studies were performed according to NCCLS Tentative Guideline, EP5-T.

3. Linearity: Linear to 500 U/L at 37 °C. Performed according to NCCLS Guideline EP6-P.
4. Sensitivity: Based on an instrument resolution of $A = 0.001$, the method presented shows a sensitivity of 1.8 U/L.

REFERENCES

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4. Bergmeyer, H.U. *Principles of Enzymatic Analysis*. Verlag Chemic, 1978.
5. Young D.S. *Effects of drugs on clinical laboratory tests*. AACC Press, Washington D.C., 1990.
6. Henry JB. *Clinical Diagnosis and Management by Laboratory Methods*, 17th ed. WB Saunders Co., 1984, p 1437.