

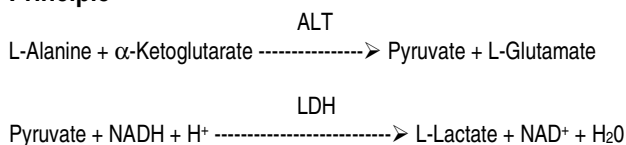
Intended Use

For the quantitative determination of alanine aminotransferase in serum.

Method History

UV methods for ALT determination were described by Henley¹ in 1955 and Wroblewski and La Due² in 1956. The procedure was improved and optimized by Henry et al³ in 1960. In 1974 the Scandinavian Society for Clinical Chemistry⁴ recommended optimized reaction conditions. The International Federation of Clinical Chemistry (IFCC)⁵ published a proposed recommended method in 1980 utilizing the LDH-NADH coupled assay. The procedure described herein is based on that method.

Principle



ALT catalyzes the transfer of the amino group from L-alanine to α -ketoglutarate resulting in the formation of pyruvate and L-glutamate. Lactate dehydrogenase catalyzes the reduction of pyruvate and the simultaneous oxidation of NADH to NAD. The resulting rate of decrease in absorbance is directly proportional to ALT activity.

Reagents

(Concentrations refer to reconstituted reagent):

α -Ketoglutaric Acid 13mM, D-Alanine 400 mM, NADH 0.2 mM, LDH 1200 U/L, Tris Buffer, pH 7.5 \pm 0.1, non-reactive fillers and stabilizers.

Reagent Preparation

Reconstitute vial with the volume of distilled water stated on the vial label. Swirl gently until reagent is completely in solution.

Reagent Storage

1. Store dry reagent at 2-8°C (refrigerated).
2. Reconstituted reagent is stable for thirty days if immediately refrigerated and for 48 hours at room temperature.

Reagent Deterioration

The reagent should not be used if the reconstituted reagent has an initial absorbance less than 0.8 at 340 nm.

Precautions

This reagent is for *in vitro* diagnostic use only.

Specimen Collection and Storage

1. Hemolyzed samples cannot be used as red cells contain ALT.⁶
2. ALT in serum is stable for three days at room temperature, seven days refrigerated, and thirty days frozen.⁶

Interferences

A number of drugs and substances affect ALT activity. See Young, et al.⁷

Materials Provided

ALT (SGPT) reagent.

Materials Required but not Provided

1. Accurate pipetting devices
2. Test tubes/rack
3. Timer
4. Spectrophotometer able to read at 340 nm (UV)
5. Heating bath/block (37°C)

Procedure (Automated)

Refer to specific instrument application instructions.

Procedure (Manual)

1. Reconstitute reagent according to instructions.
2. Pipette 1.0ml of reagent into appropriate tubes and prewarm at 37°C for five minutes.
3. Zero spectrophotometer with water at 340nm.
4. Transfer 0.10ml (100ul) of sample to reagent, mix and incubate at 37°C for one minute.
5. After one minute, read and record absorbance. Return tube to 37°C. Repeat readings every minute for the next two minutes.
6. Calculate the average absorbance difference per minute (Δ abs./min.).
7. The Δ abs./min. multiplied by the factor 1768 (see calculations) will yield results in IU/L.
8. Samples with values above 500IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Procedure Notes

1. 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.
2. 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 0.05ml (50ul) of sample and multiplying the final answer by two.
3. A very low final reading, together with a small absorbance change between readings could indicate a very high ALT level. Dilute and re-assay as in step #8.

Calibration

The procedure is standardized by means of the millimolar absorptivity of NADH taken as 6.22 at 340nm under the test conditions described.

Calculations

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

$$\text{ALT (IU/L)} = \frac{\Delta\text{Abs./Min.} \times 1.10 \times 1000}{6.22 \times 0.10 \times 1.0} = \Delta\text{Abs./min.} \times 1768$$

Where Δ Abs./Min. = Average absorbance change per minute
1.10 = Total reaction volume (ml)
1000 = Conversion of IU/ml to IU/L
6.22 = Millimolar absorptivity of NADH
0.10 = Sample Volume (ml)
1.0 = Light path in cm

ALT (SGPT) Reagent Set

Example: If the average absorbance change per minute = 0.12 then

$$0.12 \times 1768 = 212 \text{ IU/L}$$

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.

Quality Control

The validity of the reaction should be monitored by use of control serums with known normal and elevated values for ALT.

Expected Values⁸

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

1. Linearity: 500 IU/L
2. Comparison: Studies between the present method and a similar method yielded a correlation coefficient of 0.999 and a regression equation of $y=0.95x-3.6$
3. Precision:

Within Run			Run to Run		
<u>Mean</u>	<u>S.D.</u>	<u>C.V.%</u>	<u>Mean</u>	<u>S.D.</u>	<u>C.V.%</u>
25	2.1	8.4	28	1.8	6.4
84	2.3	2.7	84	3.0	3.6

References

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