

Intended Use

For the quantitative determination of Alanine Aminotransferase in serum.

Clinical Significance

ALT is widely distributed in tissues with the highest concentrations found in the liver and kidneys. Even so, ALT is considered more liver-specific than AST. Elevated levels of ALT are often only observed in liver diseases such as cirrhosis, hepatitis, or metastatic carcinoma. However, there can be elevated levels of ALT with infectious mononucleosis, muscular dystrophy, and dermatomyositis.¹

Method History

UV methods for ALT determination were described by Henley² in 1955 and Wroblewski and La Due³ in 1956. The procedure was improved and optimized by Henry et al⁴ in 1960. In 1974, the Scandinavian Society for Clinical Chemistry⁵ recommended optimized reaction conditions. The International Federation of Clinical Chemistry (IFCC)⁶ 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

After combining R1 and R2 the reagent contains: L-alanine 500mM, α -ketoglutaric acid 15mM, LDH(microbial) >2000IU/L, NADH >0.18mM, Buffer 100mM, pH 7.5±0.1, Sodium azide 0.25%, Stabilizers.

Reagent Preparation

Prepare working reagent by mixing 5 parts of R1 reagent with 1 part R2 reagent. (e.g. 250 ul R1 with 50 ul R2 reagent.)

Reagent Storage

1. Store reagents at 2-8°C.
2. Working reagent is stable for 48 hours at room temp. (15-30°C) and for 14 days when refrigerated (2-8°C).

Reagent Deterioration

Do not use reagent if:

1. The initial absorbance at 340nm is below 0.800.
2. The reagent fails to meet stated parameters of performance.

Precautions

1. This reagent set is for *in vitro* diagnostic use only.
2. The reagent contains sodium azide (0.25%) as a preservative. Do not ingest. May react with lead and copper plumbing to form highly

explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

Specimen Collection and Storage

1. Hemolyzed samples cannot be used as red cells contain ALT.⁷
2. ALT in serum is stable for three days at room temperature (15-30°C), seven days refrigerated (2-8°C), and thirty days frozen (-20°C).⁷

Interferences

1. A number of drugs and substances affect ALT activity. See Young, et al.⁸
2. Bilirubin to at least 30 mg/dl, and hemoglobin to at least 400 mg/dl, have been found to have a negligible effect on this procedure.

Materials Provided

ALT (SGPT) Reagents R1 and R2.

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).

Test Procedure (Automated)

Wavelength:	340 nm
Assay Type:	Kinetic
Sample/Reagent Ratio:	1:10
Reaction Direction:	Decreasing
Temperature:	37°C
Lag Time:	60 seconds
Read Time:	60 seconds
Low Normal:	5 U/L
High Normal:	34 U/L

Application Parameters for various automated instruments are available. Please contact the Technical Service Department for specific information.

Procedure (Manual)

1. Reconstitute reagent according to instructions.
2. Pipette 1.0ml of reagent into appropriate tubes and pre-warm 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 mean absorbance difference/minute (Δ Abs./Min.).
7. The Δ Abs./Min. multiplied by the factor 1768 (See Calculation) will yield results in IU/L.

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. 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 necessary.

Liquid ALT (SGPT) Reagent Set

Limitations

1. 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.
2. Samples with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Calibration

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

Calculation

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

Example: If the average absorbance change per minute = 0.12 then $0.12 \times 1768 = 212$ IU/L

NOTE: If test parameters are altered the factor has to be recalculated using the above formula.

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

Quality Control

The validity of the reaction should be monitored using control sera with known normal and abnormal ALT (SGPT) values. These controls should be run at least with every shift in which ALT (SGPT) assays are performed. It is recommended that each laboratory establish their own frequency of control determination.

Expected Values⁹

4 to 24 IU/L (30°C)

4 to 36 IU/L (37°C)

Since the expected values are affected by age, sex, diet, and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

Performance

1. Linearity: 0-500 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 $y=0.96x + 3.2$. (n=128, range=7-625 IU/L)

3. Precision:

Within Run (n=20)			Run to Run (n=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
30	0.7	2.3	31	0.7	2.3
116	0.8	0.7	118	1.7	1.4
381	3.1	0.8	382	2.5	0.9

4. Sensitivity: The sensitivity for this reagent was investigated by reading the change in absorbance at 340nm for a saline sample and serums with known concentrations. Ten replicates were performed. The results of this investigation indicated that, on the analyzer used, the ALT (SGPT) reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 U/L ALT activity gives a $\Delta\text{Abs./Min.}$ of 0.0004.

References

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