

Intended Use

For the quantitative determination of Alanine Aminotransferase (ALT) in serum using the Yumizen C230 and Yumizen C240 analyzers. **Rx Only.**

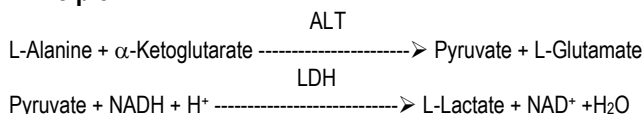
Clinical Significance

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 >450mM, α -ketoglutaric acid >14mM, LDH(microbial) >2000IU/L, NADH >0.18mM, buffer, sodium azide 0.28%, Stabilizers.

Reagent Preparation

The reagents are ready to use.

Reagent Storage

Store the reagents at 2-8°C. The reagent is stable until the expiration date appearing on the label when stored as directed.

Reagent Deterioration

Do not use reagent if:

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

Precautions

- This reagent set is for *in vitro* diagnostic use only.
- The reagent contains sodium azide (0.28%) 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

- Hemolyzed samples cannot be used as red cells contain ALT.⁷
- 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

- A number of drugs and substances affect ALT activity. See Young, et al.⁸
- 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

- Yumizen C230 / Yumizen C240 Analyzer
- Yumizen C230 / Yumizen C240
- Chemistry control, catalog number C7592-100

Test Parameters

Test:	ALT	Chemistry:	ALT
Chemistry No.:	202	Print Name:	ALT
Reaction Type:	Kinetic	Reaction Direction:	Negative
Pri. Wave:	340 nm	Sec. Wave:	405 nm
Decimal.:	0	Samp. Type:	Serum
Blank Time:		Reaction Time:	3 11
Unit:	U/L	Incubation Time:	3

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard;	9	uL	uL	uL R1: 180	uL
Decreased;		uL	uL	uL R2: 45	u/L
Increased;		uL	uL	uL	u/L

Linearity Range (Standard):	0-300	Linearity Limit:	0.3
Linearity Range (Decreased):		Substrate Depletion:	5000
Linearity Range (Increased):		Mixed Blank Abs.:	- 40000 40000
R1 Blank Abs.:	- 40000 40000	On-board Stability:	30 Day (s)
Blank Response	- 40000 40000	Reagent Alarm Limit:	5
Twin Chemistry:			

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:	
Range:	Flag:

Slope Offset:			
Slope	Offset	Unit	
1	0	U/L	

Pretreatment:	
Pretreat Sample Vol.:	uL
Pretreat Reagent Vol.:	uL

Ref. Range:				
Sample Type:	Gender:	Age Range:	Ref. Range:	Critical Range: Unit:

Calibration Setup Parameters

Chem:	ALT			
Calibration Setting				
Math Model:	K Factor			
Factor:	4700.000	Replicates:	2	
Acceptance Limits				
Cal Time:	24 hr.			
Slope Diff:	SD:			
Sensitivity:	Repeatability			* User Defined
Deter Coeff:				
Auto Calib.	<input type="checkbox"/> Cal Time			

Pointe 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 (Example)

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 its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

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: A study was performed between the Yumizen 200 series and a similar analyzer using this method, resulting in a correlation coefficient of 0.999 and a regression equation of $y=0.94x + 5.8$. (n=33).
3. Precision: Precision studies were performed using the Yumizen 200 series analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.¹⁰

Within Run (n=20)

Mean	S.D.	C.V.%
35.4	1.3	3.6
89.5	1.1	1.3

Day to Day (n=20)

Mean	S.D.	C.V.%
41.8	1.5	3.6
111.9	2.6	2.3

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 IU/L ALT activity gives a $\Delta\text{Abs./Min.}$ of 0.0004.

References

1. Tietz, N.W., Fundamentals of Clinical Chemistry, W.B. Saunders co., p 674 & 675 (1982).
2. Henley, K.S., Pollard, H.M., J. Lab. Clin. Med. 46:785 (1955).
3. Wroblewski, F., La Due, J.S., Proc. Soc. Exp. Biol. Med. 91:569 (1956).
4. Henry, R.J., et al, Am. J. Clin. Path. 34:381 (1960).
5. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, Scand. J. Clin. Lab. Invest 32:291 (1974).
6. Clinica Chimica Acta 105:145F-172F (1980).
7. Henry, R.J., Clinical Chemistry: Principles and Technics, Harper & Row, NY, P522 (1968).
8. Young, D.S., et al, Clin. Chem. 21:1D (1975).
9. Henry, J.B., Clinical Diagnosis & Management by Laboratory Methods, W.B. Saunders Co., Philadelphia, P1437 (1984).
10. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Symbol Key

Use by (YYYY-MM-DD)	Lot and batch code
Catalog number	Manufacturer
In vitro diagnostic medical device	Temperature limitation
Consult instructions for use	Rx Only: Prescription Use Only
CE mark	Authorized representative in the European Community

12-A7526-100 Manufactured by HORIBA Instruments Incorporated - Pointe Brand 5449 Research Drive Canton, MI 48188

Manufactured by HORIBA Instruments Incorporated – Pointe Brand
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Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.