

Intended Use

For the quantitative determination of Alanine Aminotransferase (ALT) in serum using the Yumizen C560 analyzer. **Rx Only.**

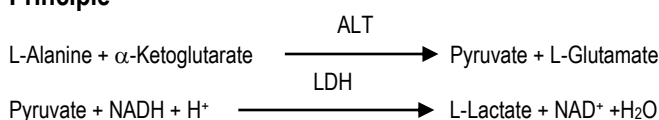
Clinical Significance

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. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

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 and Hazards

1. This reagent set is for *in vitro* diagnostic use only.
2. 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.

Hazards:

R1: Hazard Classifications: Not a hazardous substance or mixture.

Pictogram: Not required.

Signal Word: Not required.

Hazard Statements: Not a hazardous substance or mixture.

Precautionary Statements: Not a hazardous substance or mixture.

R2: Hazard Classifications: Acute Toxicity, Dermal (Category 4)

Hazard Statements: H312: Harmful in contact with skin

Precautionary Statements: Prevention:

P280 Wear protective gloves/protective clothing/eye protection/face protection. **Response:** P312 Call a POISON CENTER or

doctor/physician if you feel unwell. P363 Wash contaminated clothing before reuse. P302 + P352 IF ON SKIN: wash with plenty of soap and water. **Disposal:** P501: Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations. **Refer to the Safety Data Sheet for this product (SDS-A7526) available by calling 1-734-487-8300.**



Signal Word: Warning

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.

Pointe ALT Reagent Set

Materials Provided

ALT (SGPT) Reagents R1 and R2, 14-A7526-375

Materials Required but not Provided

1. Yumizen C560 Analyzer
2. Yumizen C560 Operation manual
3. Chemistry control, catalog number C7592-100

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.

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. Assay Range: 3-500 IU/L.
2. Comparison: A study was performed between the Yumizen C560 and a similar analyzer using this method, resulting in the following:

Method	ALT
N	85
Mean ALT (IU/L)	62.7
Range (IU/L)	8-461
Standard Deviation	95.9
Regression Analysis	$y = 1.080x + 2.1$
Correlation Coefficient	0.9982

3. Precision: Precision studies were performed using the Yumizen C560 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.¹⁰

Sample	Within Day		
	LOW	MID	HIGH
N	20	20	20
Mean	62.4	146.7	349.6
Standard Deviation	1.1	1.2	1.6
Coefficient of Variation (%)	1.7%	0.8%	0.5%

Sample	Total		
	LOW	MID	HIGH
N	40	40	40
Mean	57.3	142.2	345.9
Standard Deviation	1.2	1.9	4.1
Coefficient of Variation (%)	2.1%	1.3%	1.2%

4. Sensitivity: Sensitivity: 2SD limit of detection (95% Conf) = 3 IU/L

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).

CHEMISTRY PARAMETERS

Chem:	ALT	No.:	202	Sample Type:	Serum
Chemistry:	Alanine Transaminase	Print Name:	ALT	Reaction Direction:	Negative
Reaction Type:	Kinetic	Sec Wave:	412	Decimal	0
Pri Wave:	340	Reaction Time:	56	71	
Unit:	U/L	Reagent Vol.	Diluent		
Blank Time:	0	0			
	Sample Vol.	Aspirated	Diluent	R1:	120 ul
Standard:	6.0 ul	--- ul	--- UI	R2:	--- ul
Decreased:	--- ul	--- ul	--- UI	R3:	--- ul
Increased:	--- ul	--- ul	--- UI	R4:	--- ul
	<input type="checkbox"/> Sample Blank	<input checked="" type="checkbox"/> Auto Rerun			
Slope/Offset Adjustment					
	Slope: 1	Offset: 0			

Linearity Range (Standard)	3	500	Linearity Limit:	0.3
Linearity Range (Decreased)	---	---	Substrate Depletion:	5000
Linearity Range (Increased)	---	---	Mixed Blank Abs:	
R1 Blank Abs:	---	---	Uncapping Time	
Blank Response:	---	---	Reagent Alarm Limit:	
Twin Chemistry:			<input type="checkbox"/> Enzyme Linear Extension	
<input type="checkbox"/> Prozone Check		<input type="radio"/> Rate Check	<input type="radio"/> Antigen Addition	
Q1:	Q2:	Q3:	Q4:	
PC:	ABS:			

Pointe ALT Reagent Set

CALIBRATION PARAMETERS

Calibrator Definition						
Calibrator:	*	Lot No.:	*			
Exp Date:	*					
Carousel		Pos				
Sample Carousel 1	*					
Sample Carousel 2						
Sample Carousel 3						
Reagent/Calibration						
<u>Calibrator</u>	<u>Pos</u>	<u>Lot No</u>	<u>Exp Date</u>	<u>Chem</u>	<u>Conc</u>	<u>Unit</u>
Water	W	*	*	ALT	0	U/L
Calibration Setup						
Chem:	ALT					
<u>Calibration Settings</u>						
Math Model:	K Factor					
Factor:	4700	Replicates:	1			
<u>Acceptance Limits</u>						
Cal Time:	24	Hour				
Slope Diff:	---	SD:	---			
Sensitivity :	---	Repeatability:	---			
Deter Coeff:	---					
<u>Auto Calib.</u>						
<input type="checkbox"/> Bottle Changed	<input type="checkbox"/> Lot Changed	<input type="checkbox"/> Cal Time				

It is recommended that two levels of control material be assayed daily.

* Indicates user defined parameter.

REF 14-A7526-375



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



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.

Manufactured by HORIBA Instruments Incorporated – Pointe Brand
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Symbol Key

Use by (YYYY-MM-DD) **LOT** Lot and batch code **REF** Catalog number
 Manufacturer Temperature limitation Consult instructions for use
IVD In vitro diagnostic medical device **Rx Only:** Prescription Use Only