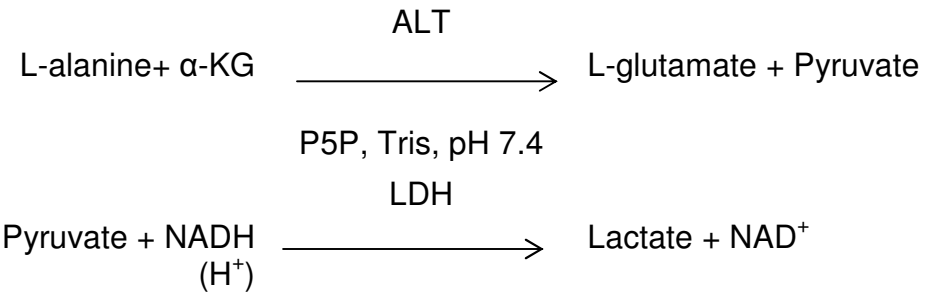
Alanine Aminotransferase

Principle

Alanine aminotransferase catalyzes the transamination of L-alanine to α-ketoglutarate (α-KG), forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of reduced nicotinamide-adenine dinucleotide (NADH). The change in absorbance is directly proportional to the alanine aminotransferase activity and is measured using a bichromaic (340, 700 nm) rate technique.



Clinical Significance

I**ntended Use:** The ALT method is an *in vitro* diagnostic test for the quantitative measurement of alanine aminotransferase in human serum and plasma on the Dimension Vista® System.

Summary: The alanine aminotransferase method is an adaptation of the recommended procedure of the IFCC as described by Bergmeyer.[1] The procedure is based on the principles outlined by Wroblewski and LaDue[2] but is modified to contain pyridoxal-5-phosphate (P5P) as an activator and to replace phosphate buffer with tris (hydroxymethyl) aminomethane. Significant elevations of ALT are found in diseases of the liver, such as hepatitis, necrosis, jaundice and cirrhosis. ALT levels can be elevated even before clinical jaundice appears.[3]

Specimen

Order # 14456

Specimen type:	Serum and plasma (lithium heparin), collected according to lab policy. Serum/plasma should be separated from cells as soon as possible with a maximum limit of two hours from time of collection, and should be free of particulate matter.
Required Volume	0.5mL serum/plasma preferred. Minimum volume: 0.2 mL
Storage/Stability	2-8°C for 7 days; <-20°C for one month. Avoid repeat freezing and thawing.

	Thawed frozen specimens which are turbid must be clarified by centrifugation prior to testing.

Reagents

ALTI Flex reagent cartridge, Catalog No. K2143

Wells[a]	Form	Ingredient	Concentration[b]	Source
1 – 2	Liquid	Tris buffer	1200 mmol/L	
3 – 6	Tablet[c]	LDH	7712 U/L	Porcine muscle
		NADH	0.57 mmol/L	
		P5P	0.39 mmol/L	
7 – 10	Tablet[c]	α-KG	61 mmol/L	
11 – 12	Liquid	Alanine	1170 mmol/L	

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Nominal value in hydrated cartridge.
c. Tablet contains excipients.

Risk and Safety:

Irritant. Contains 2-chloroacetamide.
May cause sensitization by skin contact.
Avoid contact with skin.
Wear suitable gloves.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

Precautions: Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.

For *in vitro* diagnostic use.

Reagent Preparation: Hydrating, diluting and mixing are automatically performed by the Dimension Vista® System.

Store at: 2 – 8 °C

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed wells on the instrument are stable for 30 days.

Open Well Stability: 10 days for wells 1 – 2, 11 – 12
5 days for wells 3 – 10

Instrumentation/Equipment

Dimension Vista 1500

Calibration

Calibration Material ENZ 2 CAL, Cat. KC321
Calibration Scheme 2 levels, n=5

Units	U/L
Typical Calibration Levels	Level 1 (Calibrator A): 0 U/L Level 2 (Calibrator B): 1050 U/L
Calibration Frequency	Every 90 days for any one lot Calibration interval may be extended based on acceptable verification of calibration.
A new calibration is required:	• For each new lot of Flex® reagent cartridges • After major maintenance or service, if indicated by quality control results • As indicated in laboratory quality control procedures • When required by government regulations

Quality Control

At least once each day of use, analyze two levels of a Quality Control (QC) material with known alanine aminotransferase activity. Follow the laboratory's internal QC procedures if the results obtained are outside acceptable limits.

Procedure

Test Steps

Sampling, reagent delivery, mixing, and processing are automatically performed by the Dimension Vista® System. For details of this processing, refer to your Dimension Vista® Operator's Guide.

Test Conditions

Sample Volume (delivered to the cuvette)	14.6 µL
Reagent 1 Volume	58.3 µL
Reagent 2 Volume	49.2 µL
Temperature	37.0 ℃
Reaction Time	5.6 minutes
Wavelength	340 and 700 nm
Type of Measurement	Bichromatic Rate

Reporting Results

The instrument calculates the activity of ALT in U/L using the calculation scheme described in your Dimension Vista® Operator's Guide.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Analytical Measurement Range (AMR): 6 – 1000 U/L

Clinical Reportable Range (CRR): 6 – 20,000 U/L

This is the range of analyte values that can be measured directly from the specimen without any dilution or pretreatment that is not part of the usual analytical process and is equivalent to the assay range.

- Samples with results in excess of 1000 U/L should be repeated on dilution.

  Use Autodilution (AD): The autodilute sample volume is 4.16 µL (dilution factor = 3.5) for serum/plasma. Special dilutions x 10 and x20 are also available. Refer to your Dimension Vista® Operator's Guide.

  Manual Dilution: Dilute with Enzyme Diluent, Cat. No. 790035901, to obtain results within reportable range. Use x10 dilution. Enter dilution factor on the instrument. Reassay. Resulting readout is corrected for dilution.

- Samples with results less than 6 U/L will be reported as "less than 6 U/L" by the instrument.

Procedural Notes/Problem-Solving Tips

Limitations of Procedure

The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in ALT results. Refer to your Dimension Vista® Operator's Guide for the meaning of report flags and comments. Any report containing flags and/or comments should be addressed according to your laboratory's procedure manual and not reported.

Interfering Substances

1. Bilirubin (conjugated) at 40 mg/dL [684 µmol/L] decreases ALTI results at an activity of 70 U/L [1.17 µkat/L] by -12%.
2. Bilirubin (conjugated) at 60 mg/dL [1026 µmol/L] decreases ALTI results at an activity of 144 U/L [2.40 µkat/L] by -13%.
3. Triglycerides above 400 mg/dL [4.52 mmol/L] tripped a test report message; therefore the magnitude of the interference could not be determined.
4. Lipemia (Intralipid®) of 600 mg/dL [6.78 mmol/L] and above tripped a test report message; therefore the magnitude of the interference could not be determined.
5. Intralipid® is a registered trademark of Fresenius Kabi AG, Bad Homburg, Germany.

Expected Values: 15 – 60 U/L

The reference interval was calculated from in-house studies.

Maximum Observed Repeatability

The expected maximum observed standard deviations for repeatability (within-run precision)

using n=5 replicates at the following ALTI activity are:

ALTI Activity	Acceptable SD Maximum
24 U/L [0.40 µkat/L]	2.3 U/L [0.04 µkat/L]
100 U/L [1.67 µkat/L]	4.8 U/L [0.08 µkat/L]

A system malfunction may exist if the acceptable SD maximum is exceeded.

Specific Performance Characteristics

The following data represent typical performance for the Dimension Vista® System.

Precision[8, e]

Material	Mean U/L [µkat/L]	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool 1	38 [0.64]	0.7 [0.01] (1.8)	1.9 [0.03] (4.9)
Serum Pool 2	143 [2.39]	1.4 [0.02] (1.0)	4.1 [0.07] (2.8)
Plasma Pool 1	139 [2.32]	1.1 [0.02] (0.8)	4.8 [0.08] (3.5)
Bio-Rad Multiqual® Control			
Level 1	24 [0.40]	0.6 [0.01] (2.6)	1.1 [0.02] (4.8)
Level 2	77 [1.28]	1.0 [0.02] (1.4)	1.4 [0.02] (1.9)
Level 3	175 [2.93]	1.7 [0.03] (1.0)	3.4 [0.06] (1.9)
MAS® chemTRAK® H Control			
Level 1	24 [0.40]	0.5 [0.01] (2.3)	0.7 [0.01] (2.9)
Level 2	100 [1.67]	1.1 [0.02] (1.1)	2.9 [0.05] (2.9)
Level 3	205 [3.43]	1.8 [0.03] (0.9)	2.1 [0.03] (1.0)

e. CLSI/NCCLS EP5-A2 was used. During each day of testing, two separate runs, with two test samples, for each test material, were analyzed for 20 days.

Multiqual® is a registered trademark of Bio-Rad Laboratories, Irvine, CA 92618, USA.

MAS® and chemTRAK® are registered trademarks of Medical Analysis Systems Inc.,Camarillo, CA 93012-8058, USA.

Method Comparison[9]

Regression Statistics[f]

Comparative Method	Slope	Intercept U/L [µkat/L]	Correlation Coefficient	n
Siemens ADVIA® 1650 ALT	1.02	1.20 [0.02]	0.9996	118[g]
Dimension® ALTI	1.00	-1.26 [-0.02]	0.9996	118[g]

f. CLSI/NCCLS EP9-A2 was used. The method used to fit the linear regression line was ordinary least squares.

g. The range of 118 values in the correlation study was 9 – 867 U/L [0.15 – 14.47 µkat/L].

Specificity

Hemolysis, Icterus, Lipemia (HIL) Interference

The ALTI method was evaluated for interference according to CLSI/NCCLS EP7-A2.[10] Bias is the difference in the results between the control sample (without the interferent) and the test sample (contains the interferent) expressed in percent. Bias exceeding 10% is considered interference.

Substance Tested	Substance Concentration	ALTI Activity U/L [µkat/L]	Bias* %
Hemoglobin (hemolysate)	1000 mg/dL [0.62 mmol/L]	46 [0.77]	<10
	1000 mg/dL [0.62 mmol/L]	132 [2.20]	<10
Bilirubin (unconjugated)	80 mg/dL [1368 µmol/L]	68 [1.14]	<10
	80 mg/dL [1368 µmol/L]	138 [2.30]	<10
Bilirubin (conjugated)	30 mg/dL [513 µmol/L]	70 [1.17]	<10
	40 mg/dL [684 µmol/L]	144 [2.40]	<10
Lipemia (Intralipid®)	200 mg/dL [2.26 mmol/L]	47 [0.78]	<10
	200 mg/dL [2.26 mmol/L]	135 [2.25]	<10
	600 mg/dL [6.78 mmol/L]	47 [0.78]	---[h]
	600 mg/dL [6.78 mmol/L]	135 [2.25]	---[h]

*** Analyte results should not be corrected based on this bias.**

h. The interference testing at this level and above tripped a test report message; therefore the magnitude of the interference could not be determined.

Non-Interfering Substances

The following substances do not interfere with the ALTI method when present in serum and plasma at the activity indicated. Inaccuracies (biases) due to these substances are less than 10% at ALTI activity of 55 U/L [0.92 µkat/L] and 165 U/L [2.8 µkat/L].

Substance	Test Concentration	SI Units
Acetaminophen	20 mg/dL	1324 µmol/L
Amikacin	8 mg/dL	137 µmol/L
Ampicillin	5.3 mg/dL	152 µmol/L
Ascorbic Acid	6 mg/dL	342 µmol/L
Caffeine	6 mg/dL	308 µmol/L
Carbamazepine	3 mg/dL	127 µmol/L
Chloramphenicol	5 mg/dL	155 µmol/L
Chlordiazepoxide	1 mg/dL	33.3 µmol/L
Chlorpromazine	0.2 mg/dL	6.27 µmol/L
Cholesterol	503 mg/dL	13 mmol/L
Cimetidine	2 mg/dL	79.2 µmol/L

Substance	Test Concentration	SI Units
Creatinine	30 mg/dL	2652 µmol/L
Dextran 40	6000 mg/dL	1500 µmol/L
Diazepam	0.51 mg/dL	18 µmol/L
Digoxin	6.1 ng/mL	7.8 nmol/L
Erythromycin	6 mg/dL	81.6 µmol/L
Ethanol	400 mg/dL	86.8 mmol/L
Ethosuximide	25 mg/dL	1770 µmol/L
Furosemide	6 mg/dL	181 µmol/L
Gentamicin	1.0 mg/dL	21 µmol/L
Heparin	3 U/mL	3000 U/L
Ibuprofen	50 mg/dL	2425 µmol/L
Immunoglobulin G (IgG)	5 g/dL	50 g/L
Lidocaine	1.2 mg/dL	51.2 µmol/L
Lithium	2.2 mg/dL	3.2 mmol/L
Nicotine	0.1 mg/dL	6.2 µmol/L
Penicillin G	25 U/mL	25000 U/L
Pentobarbital	8 mg/dL	354 µmol/L
Phenobarbital	10 mg/dL	431 µmol/L
Phenytoin	5 mg/dL	198 µmol/L
Primidone	4 mg/dL	183 µmol/L
Propoxyphene	0.16 mg/dL	4.91 µmol/L
Protein: Albumin	6 g/dL	60 g/L
Protein: Total	12 g/dL	120 g/L
Salicylic Acid	50 mg/dL	3.62 mmol/L
Theophylline	4 mg/dL	222 µmol/L
Triglycerides	400 mg/dL	4.52 mmol/L
Urea	500 mg/dL	83 mmol/L
Uric Acid	20 mg/dL	1190 µmol/L
Valproic Acid	50 mg/dL	3467 µmol/L
Vancomycin	10 mg/dL	69 µmol/L

Limit of Detection and Limit of Blank[11, i]

The Limit of Detection (LoD) for ALTI is 6 U/L [0.10 µkat/L], determined consistent with CLSI guideline EP17-A[11] and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 240 determinations, with 5 blank and 5 low level samples. The Limit

of Blank (LoB) is 2.3 U/L [0.04 µkat/L].

i. LoD is the lowest concentration of analyte that can be detected reliably. LoB is the highest concentration that is likely to be observed for a blank sample.

References

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12. Siemens Vista Reagent IFU, ALT K2143, 2011-06-02. PN 781143.001-US, Siemens Healthcare Diagnostics Inc.

Medical Director Approval: Donald L Frederick, Ph.D. March 16, 2009
Name **Date**

Change of Medical Director: Robert Benirschke, PhD **September 4, 2012**
Robert Benirschke, PhD Date

REVISION HISTORY			
Rev	**Description of Change**	**Author**	**Effective Date**
0	Initial Release	DL Frederick	03/16/2009
0.1	Added revised CRR	DL Frederick	02/01/2010
0.2	Updated LIS Order Code due to HLAB install. Revised Open well stability, reagent concentrations, and test conditions per K2043 IFU update 4/26/2010	T. Mikolajczyk	1/11/2011
0.3	Revised Calibration Material per K2043 IFU update 05-13-2011, p. 2.	T King	12/14/2011
0.4	Change to new revised ALTI reagent flex. Calibration material changed to KC321 ENZ 2 Cal. Revised CRR upper concentration to 20000 U/L per x20 dilution factor available. Interfering substances, maximum observed reproducibility, and specimen performance characteristics all revised to reflect new/current ALTI package insert.	T King	12/20/2012

Reviewed

Coordinator	Date	Medical Director	Date
	3/10/10	Donald L Frederick	2/9/10
Theresa R Mikolajczyk	1/11/2011	Donald L Frederick	12/8/10
Theresa R King	12/14/2011	Donald L Frederick	12/22/11
		Robert Benirschke, PhD	9/4/12

Theresa R King	12/20/2012	Robert Omsicker, PhD	12/20/2012