Brown Clinic Northridge
511 14th Ave. NE
Watertown, SD

Brown Clinic
506 First Ave. SE
Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Alanine Aminotransferase (ALT)

PURPOSE:

The alanine aminotransferase (ALTI) method is an *in vitro* diagnostic test for the quantitative measurement of alanine aminotransferase activity in human serum or plasma on the Dimension® clinical chemistry system. Measurements of alanine aminotransferase are used in the diagnosis and treatment of certain liver diseases and heart diseases.

SUMMARY:

The Dimension® ALTI method is an adaptation of the recommended alanine aminotransferase 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 alanine aminotransferase are found in diseases of the liver, such as hepatitis, necrosis, jaundice and cirrhosis. Alanine aminotransferase levels can be elevated even before clinical jaundice appears.[3]

PRINCIPLES OF PROCEDURE:

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 bichromatic (340, 700 nm) rate technique.

$$\text{L-alanine} + \alpha\text{-KG} \xrightarrow[\text{P5P, Tris, pH 7.4}]{\text{Alanine Aminotransferase}} \text{L-glutamate} + \text{pyruvate}$$

$$\text{Pyruvate} + \text{NADH} (H^+) \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

INSTRUMENT, REAGENTS & SUPPLIES:

Wells[a]	Form	Ingredient	Concentration[b]	Source
1, 2, 3	Tablet[c]	LDH	3000 U/L	Porcine muscle

(Reagent 1)		NADH	0.22 mmol/L
		P5P	0.15 mmol/L
4, 5, 6 (Reagent 2)	Tablet[c]	α-KG	20 mmol/L
7	Liquid	Alanine	260 mmol/L
8	Liquid	Tris buffer	100 mmol/L

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

Risk and Safety:
H317
P280, P272, P302+P352, P333+P313, P501
Warning! May cause an allergic skin reaction.
Wear protective gloves/protective clothing/eye protection/face protection.
Contaminated work clothing should not be allowed out of the workplace.
IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention.
Dispose of contents and container in accordance with all local, regional, and national regulations.
Contains: 2-Chloracetamide
Safety data sheets (MSDS/SDS) available on www.siemens.com/healthcare

Precautions:
Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion.
For *in vitro* diagnostic use

Reagent Preparation: Hydrating, diluting and mixing are automatically performed by the instrument.

REAGENT STORAGE & STABILITY:
Store at: 2 – 8 °C

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed or unhydrated cartridge wells on the instrument are stable for 30 days.
Open Well Stability: 3 days for wells 1 – 6, 30 days for wells 7 – 8

SPECIMEN REQUIREMENTS:
Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method. In the preparation of serum or plasma samples, avoid prolonged contact with separated red cells.[4]
Follow the instructions provided with your specimen collection device for use and processing.[5]
For Serum: Complete clot formation should take place before centrifugation. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.[6]

Specimen:
- Patient preparation: no preparation required

- Specimen types: serum and plasma (lithium heparin).
- Stability: Separated samples are stable for 7 days refrigerated at 2 – 8 °C. For longer storage, specimens may be frozen for 1 month at -20 °C or colder.[7] Avoid repeated freezing and thawing. Thawed frozen specimens which are turbid must be clarified by centrifugation prior to testing.

The purpose of specimen storage information is to provide guidance to the customer; however, the customer may validate their own procedures for storing patient samples.

CONTROLS:

At least once each day of use, analyze two levels of a Quality Control (QC) material with known alanine aminotransferase activity.

Follow government regulations or accreditation requirements for quality control frequency. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.

PROCEDURE:

Materials Provided

ALTI Flex® reagent cartridge, Cat. No. DF143

Materials Required But Not Provided

Enzyme II Calibrator, Cat. No. DC143

Enzyme Diluent, Cat. No. 790035901

Quality Control Materials

Test Steps

Sampling,[d,e] reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® clinical chemistry system. For details of this processing, refer to your Dimension® clinical chemistry system Operator's Guide.

d. The sample container must contain sufficient quantity to accommodate the sample volume plus dead volume. Precise container filling is not required.
e. An alternate sample size of 20 µL can be programmed; refer to the Dimension® clinical chemistry system Operator's Guide for the use of alternate sample size.

Test Conditions

Reaction Cuvette

Sample Volume (delivered to the cuvette)	Standard 35 µL Reduced 20 µL
Reagent 1 Volume	30 µL
Reagent 2 Volume	80 µL
Diluent Volume	215 µL
Temperature	37 °C
Wavelength	340 and 700 nm
Reaction Time	6.5 minutes
Type of Measurement	Bichromatic Rate

Analytical Measurement Range (AMR): 6 – 1000 U/L [0.10 – 16.70 µkat/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 [16.70 µkat/L] are reported as "Above Assay Range" and should be repeated on dilution.
  Autodilution (AD): The recommended autodilute sample volume is 10 µL (dilution factor = 3.5) for serum and plasma. Refer to your Dimension® clinical chemistry system Operator's Guide.
  Manual Dilution: Results in excess of 1000 U/L [16.70 µkat/L] should be repeated after diluting the sample with Enzyme Diluent (Cat. No. 790035901) or equivalent to produce a sample within the assay range. Enter dilution factor. Reassay. Resulting readout is corrected for dilution.
- Samples with results less than 6 U/L [0.10 µkat/L] should be reported as "less than 6 U/L [0.10 µkat/L]".

Calibration:

Assay Range	6 – 1000 U/L [0.10 – 16.70 µkat/L][f]
Calibration Material	Enzyme II Calibrator, Cat. No. DC143
Calibration Scheme	3 levels, n = 3
Units	U/L [µkat/L] (U/L x 0.0167 = µkat/L)
Typical Calibration Levels	Level 1: 0 U/L [0 µkat/L] Level 2: 500 U/L [8.35 µkat/L] Level 3: 1050 U/L [17.54 µkat/L]
Calibration Frequency	Every 90 days for any one lot
A new calibration is required	• For each 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
Assigned Coefficients	C_0 3.000 C_1 -3.375

f. Système International d'Unités [SI units] are in brackets.

Backup:
Refer to Brown Clinic Backup Policy

INTERPRETATION:

The instrument calculates the activity of alanine aminotransferase in U/L [µkat/L] using the calculation scheme illustrated in your Dimension® clinical chemistry system Operator's Guide.

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

EXPECTED VALUES:

12-78 U/L

REPORTING:

U/L

LIMITATIONS:

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 alanine aminotransferase results. Refer to your Dimension® clinical chemistry system Operator's Guide for the meaning of report flags and comments. Any result containing flags and/or comments should be addressed according to your laboratory's procedure manual.

Backup:

If the analyzer is nonfunctional, the testing will be referred to alternate Brown Clinic location or to reference laboratory for analysis.

ANALYTICAL SPECIFICITY

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

Reference: ALTI Flex® reagent cartridge insert sheet PN 717143.002 Issue Date 2015-01-30

Origination Date: 8-11-12
Date of Implementation: 9-1-12
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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-11-12_____

Approved By: _____Aaron Shives, MD_____ Date: ___10/21/2017_____
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
4/27/15	Heather Hall	Updated Information to Product Insert dated 3/14/14
6/11/15	Sam Legg	updated Risk and Safety information dated 2015/01/30
2/21/17	Lori Murray	add backup method process
7/11/17	LMurray	ref ranges verified
10/17/17	Heather Hall	Backup process modified