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

Brown Clinic
506 First Ave. SE
Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Aspartate Aminotransferase

PURPOSE:
The AST method used on the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended for the quantitative determination of aspartate aminotransferase activity in **serum** or **plasma**.

PRINCIPLE:
The aspartate aminotransferase method is an adaptation of the methodology recommended by the International Federation of Clinical Chemistry (IFCC).[1] The method uses the coenzyme pyridoxal-5-phosphate (P5P) to activate the apoenzyme and lactic acid dehydrogenase (LDH) to eliminate pyruvate interference. Significant elevations of AST are found in diseases of the liver such as hepatitis, necrosis, jaundice, and cirrhosis. AST levels can be elevated even before clinical jaundice appears.

Aspartate aminotransferase (AST) catalyzes the transamination from L-aspartate to α-ketoglutarate, forming L-glutamate and oxalacetate. The oxalacetate formed is reduced to malate by malate dehydrogenase (MDH) with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). The change in absorbance with time due to the conversion of NADH to NAD is directly proportional to the AST activity and is measured using a bichromatic (340, 700 nm) rate technique.

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightarrow[\text{pH 7.8}]{\text{AST}} \text{L-glutamate} + \text{Oxalacetate}$$

$$\text{Oxalacetate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}$$

INSTRUMENT, REAGENTS & SUPPLIES:
Reagents

Wells [a]	Form[b]	Ingredient	Concentration [c]	Source
1–3	Tablet	MDH	3000 U/L	porcine muscle
		LDH	900 U/L	

		NADH	0.21 mmol/L
1–3	Tablet	P5P	0.18 mmol/L
		Buffer	
4–6	Tablet	Aspartic acid	180 mmol/L
	(2/well)	α-ketoglutaric acid	11. 5 mmol/L

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

Precautions:

Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion.[2]

Safety Data Sheets (MSDS/SDS) can be found at www.siemens.com/healthcare

For *in vitro* diagnostic use

Reagent Preparation: Mixing and diluting are automatically performed by the Dimension® system.

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. Once wells have been entered by the instrument, they are stable for 72 hours.

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 the separated red cells.[2]

Follow the instructions provided with your specimen collection device for use and processing.

Complete clot formation should take place before centrifugation.

Specimen:
Patient preparation: none required

Specimen type: serum or heparinized plasma

Stability:

Room Temperature: 3 days
Refrigerated at 2-8 degrees C: 7 days
Frozen at -20 degrees C or colder: 1 month

CONTROLS:
At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's

laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

PROCEDURE:

Materials Required:

AST Flex® reagent cartridge, Cat. No. DF41A
Enzyme Verifier, Cat. No. DC19
Quality controls material

Test Steps

Sampling, reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® system. For details of this processing, refer to your Dimension® system manual.

Test Conditions

- Sample Size: 40 µL[f], (20 µL)[g]
- Reagent 1 Volume: 100 µL
- Reagent 2 Volume: 65 µL
- Diluent Volume: 235 µL
- Test Temperature: 37° C
- Wavelength: 340 and 700 nm
- Type of Measurement: bichromatic rate

f. The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus dead volume. Precise container filling is not required.

g. An alternate sample size of 20 µL can be programmed; refer to the Operator's Guide for the use of alternate sample size.

Backup:

Refer to Brown Clinic Backup Policy

Verification

The general verification procedure is described in the Operator's Guide (also see Appendix B). The following information should be considered when verifying the aspartate aminotransferase method:

Assay Range (@ 37° C):	0 – 1000 U/L
Reference Material:	Secondary verifiers such as Enzyme Verifier (Cat. No. DC19)
Typical Verification Levels:	50, 400, 800 U/L
Verification Scheme:	Three levels in triplicate
Verification Frequency:	Every new reagent cartridge lot
	Every 90 days for any one lot
	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

Assigned Coefficients: Standard sample size = 40 µL

C0 2.000

C1 -3.537

Alternate sample size = 20 µL

C0 -2.000

C1 -7.040

INTERPRETATION:

The instrument automatically calculates and prints the activity of aspartate aminotransferase in U/L using the calculation scheme illustrated in your Dimension® system manual.

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

EXPECTED VALUES:

15 - 37 U/L

The reference interval was calculated non-parametrically and represents the central 95% of the population. (N=245, adults)

Each laboratory should establish its own reference interval for aspartate aminotransferase as performed on the Dimension® system.

REPORTING:

report format: U/L

LIMITATIONS:

AMR: 0 - 1000 U/L.

Manual Dilution: Results in excess of 1000 U/L should be repeated after diluting the sample with Enzyme Diluent (Cat. No. 790035901) or equivalent to produce a sample result within the assay range.

Enter dilution factor. Reassay. Resulting readout is corrected for dilution.

Autodilution (AD): Refer to your Dimension® system manual.

After dilution: Samples with results less that 5 U/L should be reported as "less than 5 U/L"

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension® system manual.

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: AST Flex® reagent cartridge insert sheet PN 717041.002 Issue Date 2016-2-26 Rev. E

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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-8-12

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

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New format
8-8-12	Lori Murray	changed instrument to EXL200
5/20/15	Heather Hall	Updated information to package insert dated 07/13/2012
10/15	Lori Murray	updated reporting parameters with <5 U/L
10/17/17	Heather Hall	Update information to package insert dated 2-26-2016, Modified Backup Testing Process