Brown Clinic Northridge
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BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Amylase

PURPOSE:
The AMY method used on the Dimension EXL200 integrated chemistry system is an in vitro diagnostic test intended for the quantitative determination of amylase activity in **serum**, **plasma** and **urine**

PRINCIPLE:
The AMY method on the Dimension® system utilizes a chromogenic substrate, 2-chloro-4-nitrophenol linked with maltotriose.[1] The direct reaction of a-amylase with the substrate results in the formation of 2-chloro-4 nitrophenol, which is monitored spectrophotometrically. Amylase measurements are used primarily for the diagnosis and treatment of pancreatitis. The AMY method responds to both pancreatic and salivary amylase isoenzymes.

α-amylase (a-1, 4-glucan, 4-glucanohydrolase; EC 3.2.1.1) catalyzes the hydrolysis of a defined synthetic substrate, 2-chloro-4-nitrophenyl-a-D-maltotrioside(CNPG3), to yield 2-chloro-4-nitrophenol (CNP), 2-chloro-4-nitrophenyl-α-D-maltoside(CNPG2), maltotriose (G3) and glucose. After an incubation of 70 seconds at 37°C, the absorbance due to the formation of 2-chloro-4-nitrophenol (CNP) is measured using a bichromatic (405, 577 nm) rate technique.

Amylase
CNPG3 ---------->CNP + CNPG2 + G3 + glucose

INSTRUMENT, REAGENTS & SUPPLIES:

Wells[a]	Form	Ingredient	Concentration[b]
1–6	Liquid	CNPG3	1.24 mmol/L

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Nominal value per test at manufacture.

Precautions:

Contains sodium azide (<0.1%) as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.

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: All reagents are liquid and ready-to-use.

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.[2]

Blood collection tubes containing EDTA, Citrate and Oxalate have been reported to inhibit α-amylase and should not be used.

Corvac ® and SST ® collection tubes and tubes containing sodium fluoride do not affect the AMY method.

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

Complete clot formation should take place before centrifugation.

Specimens should be free of particulate matter.

Specimen:

- patient preparation: none required
- specimen type: serum or heparinized plasma
- stability: 7 days at room temperature

  6 months at 2-8 degrees C

  Longer storage at -20 degrees C

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, refer to the quality control policy.

PROCEDURE:
The AMY Flex® reagent cartridge, Cat. No. DF17A, is required to perform the AMY test. This test is performed on the Dimension® clinical chemistry system after the method is verified .

Test Steps

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

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

e. An alternate sample size of 10 µL can be programmed; refer to the Operator's Guide for the use of alternate sample size.

Test Conditions

- Sample Size: 14 µL (10 µL) [e]
- Reagent Volume: 220 µL
- Diluent Volume: 166 µL
- Test Temperature: 37°C
- Wavelength: 405 and 577 nm
- Type of Measurement: bichromatic rate

Verification

The general verification procedure is described in your Dimension® system manual (also see Appendix B).

The following information should be considered when verifying the amylase method:

Assay Range (@37°C): 0-650 U/L

Reference Material: Secondary verifiers such as Enzyme Verifier (Cat. No. DC19)

Suggested Verification Levels: 60, 400, 725 U/L

Verification Scheme: Three levels in triplicate

Verification Frequency: Every new reagent cartridge lot.

Every 3 months for any one lot

After major maintenance or service, if indicated by quality control results

As indicated in laboratory quality control procedures

When required by government regulations

Verification Slope Range: 0.90-1.10

Assigned Coefficients: Standard Sample size = 14 µL

C_0 0.000

C_1 5.400

Alternate Sample size = 10 µL

C_0 0.000

C_1 7.560

INTERPRETATION:

The instrument automatically calculates and prints the concentration of Amylase in U/L [µmol/L] using the calculation scheme illustrated in your Dimension® system manual. A change of 0.2 milli-absorbance units (mA) per minute corresponds to an α-amylase activity of 1 U/L at 37 degrees C.

For purposes of diagnosis and treatment, results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings

REFERENCE RANGE:

(@37°C): Serum 25-115 U/L

REPORTING:

Report format: U/L

Backup:

Refer to Brown Clinic Backup Policy

LIMITATIONS:

Analytical Measurement Range (AMR): 0-650 U/L

This is the range of analyte values that can be directly measure on 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 650 U/L should be repeated on dilution.

Manual dilution: Serum/Plasma: Make appropriate dilution with Enzyme Diluent (Cat No 790035901) or equivalent to obtain result within the assay range. Enter dilution factor.

Serum/Plasma/Urine: Reassay. Resulting readout is corrected for dilution.

Autodilution (AD)

(for serum, plasma): Refer to your Dimension® system manual.

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 followup. Refer to your Dimension® system manual.

A System malfunction may exist if the following 5-test precision is observed at the standard sample size (14 µL)

Activity	SD
50 U/L	< 4 U/L
600 U/L	<10 U/L

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.

REFERENCES:

AMY Flex® reagent cartridge insert sheet PN 717017.003 Issue Date 2016-2-26

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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	changes instrumentation to EXL200
4/15/15	Heather Hall	Updated information from new package insert dated 4-18-2012
2/21/17	lori murray	added backup method process
10/17/2017	Heather Hall	Updated information to package insert dated 2-26-2016 Modified backup method process