TRAINING UPDATE

Lab Location:	GEC	**Date Distributed:**	4/17/2015
Department:	Core	**Due Date:**	5/10/2015
		Implementation:	**5/11/2015**

DESCRIPTION OF PROCEDURE

Name of procedure:

Amylase by Dimension® Xpand Chemistry Analyzer GEC.C18 v1

Description of change(s):

Section	Reason
1,7.1	Add analyzer name
5.5	Correct first entry of ‘or’ to ‘and’
6.4, 6.6	Replace LIS with Unity Real Time
6.7	Add use of TEA for lot to lot runs
8.2	Remove Lynx, specify Xpand process
10.2	Correct to whole number
10.5	Remove code QNSR, remove use of code REP from dilutions
14.3	Add new value to Lipemia interference

The revised SOP will be implemented on May 11, 2015

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training (version 1)

Technical SOP

Title	**Amylase by Dimension® Xpand Chemistry Analyzer**
Prepared by	Leslie Barrett Date: 3/18/2011
Owner	Robert SanLuis Date: 3/18/2011

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

Form revised 2/02/2007

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Amylase	Dimension® Xpand Chemistry Analyzer	AMYL

Synonyms/Abbreviations
AMY

Department
Chemistry

Form revised 2/02/2007

2. ANALYTICAL PRINCIPLE

The AMY method on the Dimension® system utilizes a chromogenic substrate, 2-chloro-4-nitrophenol linked with maltotriose. 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 (α-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

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Heparin) Serum
Collection Container	Plasma: Green top tube Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL
Transport Container and Temperature	Collection tube or plastic vial at room temperature
Stability & Storage	Room Temperature: 7 days

Criteria		
Requirements	Refrigerated:	(2-8°C) 6 months
	Frozen:	(-20°C or colder) > 6 months
Timing Considerations	N/A	
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.	
Compromising Physical Characteristics	Gross hemolysis. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)	
Other Considerations	Allow to clot completely prior to centrifugation.	

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Amylase	Siemens, Flex® reagent cartridge, Cat. No. DF17A
Enzyme Diluent	Siemens Cat. No. 790035901

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.
Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Reagent	Amylase
Container	Reagent cartridge
Storage	Store at 2-8°C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed or unhydrated cartridge wells on the instrument are stable for 30 days.

	• Once wells 1 – 6 have been entered by the instrument, they are stable for 3 days.
Preparation	Reagents are supplied ready for use. No additional preparation is required.

Reagent	**Enzyme Diluent**
Container	Reagent vial
Storage	Store at 2-8° C
Stability	• Un-reconstituted product is stable until expiration date stamped on the vial. • Discard after 7 days following reconstitution or immediately if visible turbidity appears.
Preparation	• Remove vial from refrigerator, proceed directly to next step. • Remove stopper and volumetrically add 10 mL reagent grade water. The water should be equilibrated to room temperature. • Replace stopper and invert gently 10 times. • Let vials sit for 15 minutes, then invert gently 10 times. • Let vials sit for an additional 15 minutes. Then invert 10 times and swirl gently. • Use immediately or refrigerate at 2 – 8 °C. • Before use, allow product to come to room temperature, then invert 10 times and swirl gently.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Enzyme Verifier	Siemens Dimension®, Cat. No. DC19

5.2 Calibrator Preparation and Storage

NOTE: Date and initial all calibrators upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech (6) any special storage instructions; check for visible signs of degradation.

Calibrator	Enzyme Verifier
Preparation	• Remove vials from refrigerator and allow to stand at room temperature (22 - 28° C) for 10 to 15 minutes. • Add 2.00 ± 0.02 ml purified water. The water should be at room temperature.

	• Replace stopper, and let stand for 5 minutes. Do not invert. • Swirl vials gently for 30 seconds, then gently invert 10 times. • Let vials stand for 10 minutes, and then gently invert 10 times. • Let vial stand for additional 15 minutes. Then invert 10 times and swirl gently. • Use immediately or refrigerate at 2-8° C for future use.
Storage/Stability	• Store at 2-8° C • Un-reconstituted calibrator is stable until expiration date stamped on the box. • Assigned values are stable for 8 hours after reconstitution when vials are stoppered and stored 2-8° C.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Enzyme Verifier
Assay Range	0 – 650 U/L
Suggested calibration level	See Reagent Package Insert for lot specific assigned values in U/L
Frequency	• Every new reagent cartridge lot. • Every 3 months for any one lot. • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	Three levels.
Assigned Coefficients	C_0 0.000 C_1 5.400

5.4 Calibration Procedure

1.	From Operating Menu press F5:Process Control press F1: Calibration Enter Password press F2: SETUP and RUN
2.	Select the test method to be calibrated - if lot number is incorrect Press F1: Other Lot
3.	Enter all information on screen
4.	Press F8: QC yes/no to change to yes

5. Press F4: Assign cups If additional methods need to be calibrated, select the method.
6. Press F7: Load/run
7. Load cups into assigned position
8. Press F4: RUN

5.5 Tolerance Limits

IF……	THEN……
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis
If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Liquichek™ Unassayed Chemistry Control Levels 1 and 2	Bio-Rad Laboratories Cat. No. 691 and 692

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

Control	Liquichek Unassayed Chemistry Controls Levels 1 & 2
Preparation	Allow the frozen control to stand at room temperature (18-25°C) until completely thawed. Swirl the contents gently to ensure homogeneity. (Do not use a mechanical mixer) Use immediately. After each use, promptly replace the stopper and return to 2-8°C storage.
Storage/Stability	Open controls are stable for 15 days at 2-8°C. Unopened controls are stable until the expiration date at -20 to -70°C.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing.

Refer to the Dimension® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension® Quick Reference Guide.

6.4 Tolerance Limits

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime.
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Review Patient Data

Technologist must review each result with error messages. Refer to the Dimension Xpand® system manual "Error messages" section for troubleshooting. Check for unusual patterns, trends, or distributions in patient results (such as an unusually high

percentage of abnormal results). Resolve any problems noted before issuing patient reports.

6.6 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.7 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Dimension Xpand® System

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

- Plastic serum tubes and serum cups

- Purified water (Millipore® or equivalent)
- Calibrated pipettes and disposable tips

8. PROCEDURE

AMY Flex® reagent cartridge Cat. No. DF17A is required to perform this test.

Amylase is performed on the Dimension Xpand® System after the method is calibrated (see Reference Material in Calibration section) and Quality Controls are acceptable.

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

8.1	Instrument Set-Up Protocol
1.	For instrument set up and operation: Refer to Startup and Maintenance, Siemens Dimension® Xpand procedure.
2.	Check reagent inventory
3.	Sampling, reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® Xpand system. For details of the automated parameters, see below under “Test conditions.”

8.2	Specimen/Reagent Preparation
1.	Centrifuge the specimens.
2.	Specimens are placed in Dimension® Xpand segments for analysis by the instrument. Refer to the Sample Processing, Siemens Dimension® Xpand procedure. The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus 50 µL of dead volume. Precise container filling is not required.

8.3	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension® Xpand QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension® Xpand Operators Manual
3.	The instrument reporting system contains error messages to warn the user of specific malfunctions. Results followed by such error messages should be held for follow-up. Refer to the Dimension® Xpand system manual “Error messages” section for troubleshooting.
4.	Follow protocol in Section 10.5 “Repeat criteria and resulting” for samples with results above or below the Analytical Measurement Range (AMR). Investigate any failed delta result and repeat, if necessary.

8.3	Specimen Testing
5.	Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors.

Test Conditions	
Sample Size:	14 µL
Reagent 1 Volume:	220 µL
Diluent Volume:	166 µL
Temperature:	37° C
Wavelength:	405 and 577 nm
Type of Measurement:	Bichromatic rate

9. CALCULATIONS

The instrument automatically calculates and prints the concentration of Amylase in U/L.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results as a whole number.

10.3 Units of Measure

U/L

10.4 Clinically Reportable Range (CRR)

0 - 6500 U/L

10.5 Repeat Criteria and Resulting

All repeats must replicate the original result within the total allowable error (TEa) of the assay. Refer to TEa policy for specific information.

Values that fall within the AMR or CRR may be reported without repeat. Values that fall outside these ranges must be repeated

IF the result is …	THEN…
0 U/L	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: 0 U/L
≥650 U/L	**On Board Automated Dilution:** Results ≥650 U/L will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
>1300 U/L	**Manual Dilution:** Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 10 **Diluent:** Enzyme diluent Enter dilution factor as a whole number on the "Enter Sample Data" screen.
>6500 U/L	If the recommended dilution does not give results within the clinically reportable range, report as: ">6500 U/L-REP" Bring to the attention of your supervisor prior to releasing result.

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Message	Code
Verified by repeat analysis	Append –REP to the result.

11. EXPECTED VALUES

11.1 Reference Ranges

Age	Male/Female
Adult (>17 years):	25-115 U/L
Pediatric:	
0–30 days	0-17
31–183 days	0-42
6 – 11 months	0-80
1 –17 years	0-105

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

Form revised 2/02/2007

12. CLINICAL SIGNIFICANCE

A marked rise in serum amylase occurs in 95 percent of patients with acute pancreatitis within 2-12 hours of onset. The enzyme enters the circulation from damaged pancreatic acinar cells. The highest serum activity is present 12-72 hours after the onset and usually returns to normal in 4-8 days. Serum amylase is cleared by the kidneys, and can be detected by measuring urinary amylase activity. Acute non-pancreatic conditions, which may also elevate amylase levels, include: acute parotitis, peritonitis, small intestine obstruction, perforated peptic ulcer, rupture of a tubal pregnancy, contraction of the sphincter of Oddi following morphine administration and mesenteric thrombosis. Fractionation of amylase will frequently expedite measures to distinguish acute pancreatitis (p-amylase) from the other conditions which are characterized predominantly by salivary (s- amylase) elevations.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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 Operator's Guide.

A system malfunction may exist if the following test precision is observed:

Activity	S.D.
50 U/L	> 4 U/L
600 U/L	> 10 U/L

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0 – 650 U/L

14.2 Precision

Material	Mean U/L	Standard Deviation (%CV)	
		Within-run	Total
Serum Pool			
Low	50	0.4	0.68
High	408	1.1	3.25

14.3 Interfering Substances

Hemoglobin at 1000 mg/dL decreased AMY results of 141 U/L by 19% and decreased AMY results of 108 U/L by 12%.
Immunoglobulin G at 5 g/dL increased AMY results of 134 U/L by 32%.

Total protein at 12 g/dL increased AMY results of 134 U/L by 95%.
Lipemia at 3000 mg/dL and above tripped a test report message; therefore the magnitude of the interference could not be determined.

HIL Interference:
The AMY method was evaluated for interference from hemolysis, icterus and lipemia according to CLSI/NCCLS. EP7-P. Bias, defined as the difference between the control sample (does not contain interferent) and the test sample (contains interferent), is shown in the table below. Bias exceeding 10% is considered "interference".

Substance tested	Test Concentration SI Units	AMY Activity U/L	Bias %
Hemoglobin (hemolysate)	500 mg/dL (monomer)	141	<10
Bilirubin (unconjugated)	80 mg/dL	141	<10
Lipemia (Intralipid®)	1000, 3000 mg/dL	140	<10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available.

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

16. RELATED DOCUMENTS

1. Dimension Xpand® Clinical Chemistry System Operator's Manual
2. Calibration / Verification Siemens Dimension® Xpand procedure
3. Dimension Xpand® Cal Accept Guidelines
4. Dimension Xpand® Calibration summary
5. Sample Processing, Siemens Dimension® Xpand procedure
6. Start up and Maintenance, Siemens Dimension® Xpand procedure
7. Laboratory Quality Control Program
8. QC Schedule for Siemens Dimension Xpand®
9. Laboratory Safety Manual
10. Material Safety Data Sheets (MSDS)
11. Siemens Dimension Xpand® Limits Chart (AG.F143)
12. Quest Diagnostics Records Management Procedure
13. Dimension Xpand® System Error Messages Chart
14. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
15. Hemolysis, Icteria and Lipemia Interference (Lab policy)
16. Repeat Testing Requirements (Lab policy)
17. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
18. Current package insert AMY Flex® Reagent Cartridge DF17A

17. REFERENCES

1. Ghoshal, Amit K. and Soldin, Steven J., Evaluation of the Dade Behring Dimension® RxL: Integrated chemistry system-pediatric reference ranges. Clinica Chimica Acta 2003; 331:144
2. Package Insert, AMY Flex® Reagent Cartridge DF17A, Siemens Healthcare Diagnostics Inc., 04/18/2012.
3. Package insert, Enzyme Verifier DC19, Dade Behring, 01/2013.
4. Package insert, Liquichek Unassayed Serum Chemistry Controls, Bio-Rad Laboratories, 05/2014.
5. Package insert, Enzyme Diluent, Dimension® Clinical Chemistry System, 10/2012

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP C056.000		
000	3/26/15	1,7.1	Add analyzer name	L Barrett	R SanLuis
000	3/26/15	5.5	Correct first entry of 'or' to 'and'	L Barrett	R SanLuis
000	3/26/15	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
000	3/26/15	6.7	Add use of TEA for lot to lot runs	L Barrett	R SanLuis
000	3/26/15	8.2	Remove Lynx, specify Xpand process	L Barrett	R SanLuis
000	3/26/15	10.2	Correct to whole number	A Chini	R SanLuis

000	3/26/15	10.5	Remove code QNSR, remove use of code REP from dilutions	L Barrett, A Chini	R SanLuis
000	3/26/15	14.3	Add new value to Lipemia interference	A Chini	R SanLuis
000	3/26/15	15	Update to standard wording	L Barrett	R SanLuis
000	3/26/15	16	Update SOP titles	L Barrett	R SanLuis
000	3/26/15	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13	L Barrett	R SanLuis

19. ADDENDA

None