TRAINING UPDATE

Lab Location:	GEC	**Date Distributed:**	3/6/2018
Department:	Core Lab	**Due Date:**	3/28/2018
		Implementation:	**3/12/2018**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Alkaline Phosphatase by Dimension® Xpand Chemistry Analyzer GEC.C218 v1

Description of change(s):

Note change to QC stability, other changes are mostly format updates

Section	Reason
4,5,6	Remove individual section labeling instructions and add general one
6.2	Update thawed stability
6.4, 6.6	Replace LIS with Unity Real Time
5.3	Remove specific calibration steps and reference separate SOP
10.5	Move patient review from section 6
10.6	Remove repeat value below AMR/CRR
15	Update to new standard wording, move hazard statement from 4.2
17	Update PI dates

This revised SOP will be implemented on March 12, 2018

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

Technical SOP

Title	**Alkaline Phosphatase by Dimension® Xpand Chemistry Analyzer**
Prepared by	Ashkan Chini Date: 2/5/2014
Owner	Robert SanLuis Date: 2/5/2014

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**

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TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Alkaline Phosphatase	Dimension® Xpand Chemistry Analyzer	ALKPH

Synonyms/Abbreviations
Alk Phos, ALP

Department
Chemistry

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2. ANALYTICAL PRINCIPLE

Alkaline phosphatase catalyzes the transphosphorylation of p-nitrophenylphosphate (p-NPP) to p-nitrophenol (p-NP) in the presence of the transphosphorylating buffer, 2-amino-2-methyl-1-propanol (AMP). The reaction is enhanced through the use of magnesium and zinc ions. The change in absorbance at 405 nm due to the formation of p-NP is directly proportional to the ALP activity, since other reactants are present in non-rate limiting quantities and is measured using a bichromatic (405, 510 nm) rate technique.

$$\text{p-NPP} + \text{AMP} \xrightarrow[\text{pH 10.35 Mg/Zn}]{\text{ALP}} \text{p-NP} + \text{AMP} + PO_4$$

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	None
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Heparin) Serum
Collection Container	Plasma: Mint green top tube (PST) 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 Requirements	Room Temperature: 8 hours
	Refrigerated: (2-8°C) 7 days
	Frozen: (-20°C or colder) 6 month
Timing Considerations	Serum should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.

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Criteria	
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.
Other Considerations	Allow to clot completely prior to centrifugation.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

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.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
ALPI	Siemens, Flex® reagent cartridge, Cat. No. DF150
Enzyme Diluent	Dimension® Clinical Chemistry System, REF 790035901

4.2 Reagent Preparation and Storage

Reagent	**Alkaline Phosphatase**
Container	Reagent cartridge
Storage	Store at 2-8°C
Stability	• Stable until expiration date stamped on reagent cartridges. • Sealed or unhydrated cartridge wells on the instrument are stable for 30 days. • Open well stability: o 2 days for wells 1 – 6 o 4 days for wells 7 – 8
Preparation	Reagents are supplied ready for use. No additional preparation is required.

Reagent	**Enzyme Diluent**
Container	Manufacturer supplied vial
Storage	Store at 2-8°C before and after reconstitution

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Stability	• Un-reconstituted product is stable until expiration date on vial. • Reconstituted product is stable for 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 10.0 mL of reagent grade water. • 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 store at 2-8°C. • Before use, allow 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
ALPI CAL	Siemens Dimension®, Cat. No. DC150

5.2 Calibrator Preparation and Storage

Calibrator	ALPI CAL
Preparation	Calibrator is ready for use. No preparation is required.
Storage/Stability	• Store at 2-8°C • Unused calibrator is stable until expiration date stamped on the box. • Once cap is removed, assigned values are stable for 30 days when recapped immediately after use and stored at 2 - 8°C

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	ALPI CAL
Assay Range	10 – 1000 U/L
Suggested calibration level	See Reagent Package Insert for lot specific assigned values in U/L

Frequency	• Every new reagent cartridge lot. • Every 90 days 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 in triplicate.
Assigned Coefficients	C_0 0.000 C_1 1.000
Procedure	Refer to Calibration / Verification Siemens Dimension® Xpand procedure for specific instructions.

5.4 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

Control	Liquichek Unassayed Chemistry Controls, Level 1 and 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	**Thawed**: Alk phos will be stable for 9 ~~6~~ days at 2-8°C. **Frozen**: 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 Xpand® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension X-pand® Quick Reference Guide.

6.4 Tolerance Limits and Criteria for Acceptable QC

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 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.

6.6 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

ALPI Flex® reagent cartridge Cat. No. DF150 is required to perform this test.

Alkaline Phosphatase 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.

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.
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:	7 µL
Reagent 1 Volume:	90 µL
Reagent 2 Volume:	57 µL
Reaction Time:	7.2 minutes
Temperature:	37°C
Wavelength:	405 and 510 nm
Type of Measurement:	Bichromatic Rate

9. CALCULATIONS

The instrument automatically calculates and prints the concentration of Alkaline Phosphatase 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)

10 – 10,000 U/L

10.5 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.

10.6 Repeat Criteria and Resulting

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

Values that fall below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 10 U/L	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 10 U/L
≥ 1,000 U/L	**On Board Automated Dilution:** Results ≥ 1,000 U/L will automatically have repeat testing performed into the instrument using dilution factor of 2.3. No multiplication is necessary.

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IF the result is …	THEN…
> 2,300 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.
> 10,000 U/L	If the recommended dilution does not give results within the clinically reportable range, report as: "> 10,000 U/L -REP" Bring to the attention of your supervisor prior to releasing result.

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

11. EXPECTED VALUES

11.1 Reference Ranges

Age	Female	Male
Adult (>19 years):	38 - 136 U/L	38 - 136 U/L
Pediatric:		
16 – 19 years	82 - 169	93 - 317
14 – 15 years	103 - 283	169 - 618
12 – 13 years	141 - 499	245 - 584
10 – 11 years	169 - 657	174 - 624
7 – 9 years	218 - 499	218 - 499
4 – 6 years	191 - 450	191 - 450
1 – 3 years	185 - 383	185 - 383
7 – 11 months	101 - 431	101 - 394
4 – 6 months	125 - 449	94 - 425
1 – 3 months	125 - 547	101 - 467
8 – 30 days	107 - 474	138 - 486
0–7 days	107 - 357	121 - 351

11.2 Critical Values

None established

11.3 Standard required Messages

None established

12. CLINICAL SIGNIFICANCE

Serum alkaline phosphatase levels are of interest in the diagnosis of hepatobiliary disorders and bone disease associated with increased osteoblastic activity. Moderate elevations of alkaline phosphatase may be seen in several conditions that do not involve the liver or bone. Among these are Hodgkin's disease, congestive heart failure, ulcerative colitis, regional enteritis, and intra-abdominal bacterial infections. Elevations are also observed during the third trimester of pregnancy.

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 Xpand Operator's Guide.

A system malfunction may exist if the following 5-test precision is observed:

Concentration	S.D.
150 U/L	> 7 U/L
500 U/L	> 16 U/L

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

10 – 1000 U/L

14.2 Precision

Material	Mean U/L	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Multiqual Assayed QC			
Level 1	37	0.6(1.5)	1.6(4.2)
Level 2	157	1(0.6)	3.7(2.3)
Level 3	303	3.3(1.1)	7.1(2.4)

14.3 Interfering Substances

Triglycerides above 1500 mg/dL tripped a test report message; interference could not be determined.
Lipemia at 600 mg/dL and above tripped a test report message; interference could not be determined.

HIL Interference:
The ALPI was evaluated for interference according to CLSI/NCCLS EP7-A2. Bias is 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	Substance Concentration	ALPI	Bias %
Hemoglobin (hemolysate)	1000 mg/dL	297, 823	<10
Bilirubin (unconjugated)	80 mg/dL	300, 834	<10
Bilirubin (conjugated)	80 mg/dL	307, 856	<10
Lipemia Intralipid®	500 mg/dL, 600 mg/dL	308, 878	<10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available.

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

ALPI Flex reagent cartridge causes serious eye irritation and skin irritation. Contains: 2-amino-2-methyl-1-propanol; sulfuric acid, zinc salt, heptahydrate. Wear protective gloves/protective clothing/eye protection/face protection.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing

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. Safety Data Sheets (SDS)
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 ALPI Flex® Reagent Cartridge DF150

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, ALPI Flex® Reagent Cartridge DF150, Siemens Healthcare Diagnostics Inc., 01/30/2015.
3. Package Insert, ALPI CAL, Siemens Healthcare Diagnostics Inc., 04/2016.
4. Package Insert, Unassayed Liquichek Chemistry Controls, Bio-Rad Laboratories 01/2017.
5. Package Insert, Enzyme Diluent, Siemens Healthcare Diagnostics Inc., 10/2012.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
0	2/26/18	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
0	2/26/18	6.2	Update thawed stability	L Barrett	R SanLuis
0	2/26/18	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
0	2/26/18	5.3	Remove specific calibration steps and reference separate SOP	L Barrett	R SanLuis
0	2/26/18	10.5	Move patient review from section 6	L Barrett	R SanLuis
0	2/26/18	10.6	Remove repeat value below AMR/CRR	L Barrett	R SanLuis
0	2/26/18	15	Update to new standard wording, move hazard statement from 4.2	L Barrett	R SanLuis
0	2/26/18	17	Update PI dates	L Barrett	R SanLuis

19. ADDENDA

None