



TRAINING UPDATE

Lab Location: GEC
Department: Core

Date Distributed: 4/22/2013
Due Date: 5/13/2013

DESCRIPTION OF PROCEDURE

Name of procedure:
Alanine Aminotransferase by Dimension® Xpand Chemistry Analyzer GEC.C37.000 Xpand Limits Chart AG.F143.004
Description of change(s):
<p>New SOP for upcoming implementation of ALTI reagent (replaces SOP for reagent ALT). Changes include:</p> <ul style="list-style-type: none">• update to Enzyme II calibrator• new lower limit for CRR and AMR <p>Implementation date of the new reagent is NOT yet set. It will be communicated to you by lab management.</p>

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

Approved draft for training all sites (version 000)

Technical SOP

Title	Alanine Aminotransferase by Dimension® Xpand Chemistry Analyzer	
Prepared by	Ashkan Chini	Date: 11/8/2012
Owner	Robert SanLuis	Date: 11/8/2012

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

Annual Review		
Print Name	Signature	Date

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1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Alanine Aminotransferase	Dimension® Xpand Chemistry Analyzer	SGPT

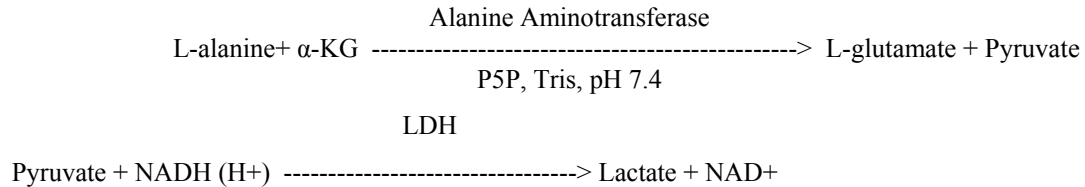
Synonyms/Abbreviations
ALT, SGPT, Included in Batteries/Packages: COMP, LIVP

Department
Chemistry

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2. ANALYTICAL PRINCIPLE

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.



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 container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours
	Refrigerated: 7 days
	Frozen: 1 month
Timing Considerations	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.

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 explanation of HMT (Specimen markedly hemolyzed)
Other Considerations	Allow Red Top or SST 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
Alanine Aminotransferase	Siemens, Flex® reagent cartridge, Cat. No. DF143
Enzyme Diluent	Siemens Diagnostics Healthcare, Inc., REF: 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	Alanine Aminotransferase
Container	Reagent cartridge
Storage	Store at 2-8° C
Stability	<ul style="list-style-type: none"> Reagent is stable until expiration date stamped on the reagent cartridges. Sealed 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. Once wells 7 - 8 have been entered by the instrument, they are stable for 30 days.

Preparation	Hydration, mixing and diluting are automatically performed by the instrument.
Reagent	Enzyme Diluent
Container	Reagent vial
Storage	Store at 2-8° C
Stability	<ul style="list-style-type: none"> • Reagent is stable until expiration date stamped on the reagent vial. • Discard after 7 days following reconstitution or immediately if visible turbidity appears.
Preparation	<ul style="list-style-type: none"> • Remove vial from refrigerator and proceed directly with next step. • Remove stopper and volumetrically add 10.0 mL of reagent grade water. • Replace stopper and invert gently 10 times. • Sit vials for 15 minutes, then invert gently 10 times. • Sit vials for an additional 15 minutes, then invert 10 times and swirl gently. • Use immediately or store at 2-8° C.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
ENZ II CAL	Siemens Dimension®, Cat. No. DC143

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	ENZ II CAL
Preparation	Calibrator is ready for use. No preparation is required.
Storage/Stability	<ul style="list-style-type: none"> • Store at 2-8° C • Unopened calibrator is stable until expiration date stamped on the box. • Opened Calibrator: once the cap is removed, assigned values are stable for 30 days when recapped immediately and stored at 2-8° C.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	ENZ II CAL
Assay Range	6 – 1000 U/L
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in U/L
Frequency	<ul style="list-style-type: none"> • 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	3 levels, n = 3

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

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6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Liquichek™ Unassayed Chemistry Control Levels 1 and 2	Bio-Rad Laboratories Cat # 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, 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	Once the control is thawed, all analytes will be stable for 15 days at 2-8°C. Unthawed 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 (notated on the QC frequency sheets posted on the instruments).

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

Step	Action
1	Acceptable ranges for QC are programmed into the Laboratory Information System (LIS), and may be posted near the instrument for use during computer downtime.
2	Run Rejection Criteria <ul style="list-style-type: none"> 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

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Step	Action
	Program to resolve the problem.
3	<p>Corrective Action:</p> <ul style="list-style-type: none"> • 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 <u>reanalyzed according to the Laboratory QC Program</u>. 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	<p>Review of QC</p> <ul style="list-style-type: none"> • 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 print-out for error messages. Refer to the Dimension® 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 the LIS. The LIS 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.

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- 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
- Calibrated pipettes and disposable tips

8. PROCEDURE

ALTI Flex® reagent cartridge Cat. No. DF143 is required to perform this test.

Alanine Aminotransferase 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.
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 Volume:	35 µL
Reagent 1 Volume:	30 µL
Reagent 2 Volume:	80 µL
Diluent Volume:	215 µL
Reaction Time:	6.5 minutes
Test Temperature:	37° C
Wavelength:	340 and 700 nm
Type of measurement:	Bichromatic rate

9. CALCULATIONS

The instrument automatically calculates the concentration of alanine aminotransferase 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)

6 – 10,000 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 listing 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...
< 6 U/L	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 6 U/L
≥ 1000 U/L	On Board Automated Dilution: Results ≥ 1000 U/L will automatically have repeat testing performed into the instrument using dilution factor of 7. No multiplication is necessary.
> 7,000 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. Reassay. Resulting readout is corrected for dilution.
> 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):	11-66 U/L	11-66 U/L
Pediatric:		
0 – 7 days	21-54	20-54
8 days – 30 days	22-46	24-54
1 – 3 months	26-61	27-54
4 – 6 months	26-51	26-55
7 – 11 months	26-55	26-59
1 – 3 years	24-59	19-59
4 – 9 years	24-49	24-49
10 – 11 years	24-44	24-49
12 – 13 years	24-44	24-68
14 – 15 years	19-44	24-59
16 – 19 years	19-49	24-54

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Measurements of alanine aminotransferase are used in the diagnosis and treatment of certain liver diseases and heart diseases. 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.

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.

The expected maximum observed standard deviations for repeatability using n = 5 replicates at the following alanine aminotransferase concentrations are:

ALTI Activity	Acceptable S.D. Maximum
50 U/L	> 3 U/L
470 U/L	> 7 U/L
870 U/L	> 11 U/L

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

6 – 1000 U/L

14.2 Precision

Material	Mean U/L	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Bio-Rad Multiqual Control			
Level 1	22	0.5 (2.2)	0.9 (4.9)
Level 2	77	0.6 (0.8)	2.0 (2.6)
Level 3	169	1.4 (0.8)	3.9 (2.3)

14.3 Interfering Substances

Bilirubin (unconjugated) at 60 mg/dL decreases ALTI results at an activity of 68 U/L by -11%.

Bilirubin (conjugated) at 40 mg/dL decreases ALTI results at an activity of 71 U/L by -13%.

Bilirubin (conjugated) at 60 mg/dL decreases ALTI results at an activity of 144 U/L by -12%.

Triglycerides above 400 mg/dL tripped a test report message; therefore the magnitude of the interference could not be determined.

Lipemia (Intralipid®) of 600 mg/dL and above tripped a test report message; therefore the magnitude of the interference could not be determined.

HIL Interference:

The ALTI method was evaluated for interference according to CLSI/NCCLS EP7-A2. 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	Substance Concentration	ALTI U/L	Bias %
Hemoglobin (hemolysate)	1000 mg/dL	49, 143	<10
Bilirubin (unconjugated)	40 mg/dL	68	<10
	80 mg/dL	140	
Bilirubin (conjugated)	30 mg/dL	71	<10
	40 mg/dL	144	
Lipemia Intralipid®	200 mg/dL	50	<10
	600 mg/dL	144	

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 X-pand® Cal Accept Guidelines
4. Dimension X-pand® 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
12. Quest Diagnostics Records Management Procedure

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- 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 Requirement (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 ALTI Flex® Reagent Cartridge DF143

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, ALTI Flex® Reagent Cartridge DF143, Siemens Healthcare Diagnostics Inc., 02/14/2012.
- 3. Package Insert, ENZ II CAL, Siemens Healthcare Diagnostics Inc., 02/2011.
- 4. Package Insert, Unassayed Liquichek Chemistry Controls, Bio-Rad Laboratories, 10/2010.
- 5. Package Insert, Enzyme Diluent, Siemens Healthcare Diagnostics Inc., 03/2008.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

DIMENSION[®] XPAND LIMITS CHART

ANALYTE	UNITS	INSTRUMENT DILUTION FACTOR	MAXIMUM RANGE AFTER ON BOARD DILUTION	MAXIMUM OFF BOARD DILUTION	CLINICALLY REPORTABLE RANGE (CRR)	DILUENT
ACTM	µg/mL	2	2.0-600.0	3	2.0-900.0	Drug Calibrator II Level 1, or Acetaminophen Free Serum
ALB	g/dL	2.5	0.6-20.0	3	0.6-24.0	Water
ALC	mg/dL	1.5	0-450	3	5-900	Water
ALP	U/L	2.3	11-2,300	10	11-10,000	Enzyme Diluent
ALTI	U/L	3.5	6 - 3,500	10	6 - 10,000	Enzyme Diluent
AMY	U/L	2	0-1,300	10	0-6,500	Enzyme Diluent
AST	U/L	8	6-8000	10	6-10,000	Enzyme Diluent
BUN	mg/dL	1.5	0-225	3	0-450	Water
CA	mg/dL	1.7	5.0-25.5	3	5.0-45.0	Water
CKI	U/L	7	7-7000	20	7-20,000	Water
CL	mmol/L	N/A	N/A	N/A	50-200	Do NOT Dilute
CREA	mg/dL	2	0.0-40.0	3	0.0-60.0	Water
CRP	mg/dL	1.5	0.2-18.0	5	0.2-60.0	Water
CTNI	ng/mL	2.5	0.04-100.00	5	0.04-200.00	Water
DBIL	mg/dL	1.9	0.0-38.0	5	0.0-100.0	Water
ECO2	mmol/L	N/A	N/A	2	5-90	Water
GLUC	mg/dL	1.5	0-750	5	0-2,500	Water
HCG	mIU/mL	200	1-200,000	5	1-1,000,000	Sample Diluent
K	mmol/L	N/A	N/A	N/A	1.0-10.0	Do NOT Dilute
LA	mmol/L	2	0.3-30.0	N/A	0.3-30.0	Do NOT Dilute
LIPL	U/L	1.5	10-2250	10	10-15,000	Water
MG	mg/dL	N/A	N/A	3	0.0-60.0	Water
MMB	ng/mL	2	0.5-600.0	5	0.5-1,500.0	Sample Diluent
NA	mmol/L	N/A	N/A	N/A	50-200	Do NOT Dilute
SAL	mg/dL	3	1.7 - 300.0	N/A	1.7 - 300.0	Do NOT Dilute
TBIL	mg/dL	2	0.0-50.0	5	0.0-125.0	Water
TP	g/dL	1.9	2.0-22.8	3	2.0-36.0	Water
TSH	µIU/mL	2	0.01-100.00	5	0.01-250.00	Sample Diluent
UCFP (CSF)	mg/dL	2	6-500	10	6-2500	Water