TRAINING UPDATE

Lab Location:	GEC	**Date Distributed:**	9/27/2017
Department:	Core	**Due Date:**	10/27/2017
		Implementation:	**10/10/2017**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Aspartate Aminotransferase by Dimension® Xpand Chemistry Analyzer GEC.C04 v3

Alanine Aminotransferase by Dimension® Xpand Chemistry Analyzer GEC.C37 v2

Description of change(s):

Most changes are updates to the format

Section	Reason
4,5,6	Remove individual section labeling instructions and add general one
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
17	Update package insert dates

* This change will be made to Xpand assays as SOPs are revised

These revised SOPs will be implemented on October 10, 2017

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

Technical SOP

Title	**Aspartate Aminotransferase by Dimension® Xpand Chemistry Analyzer**	
Prepared by	Leslie Barrett	Date: 9/3/2009
Owner	Robert SanLuis	Date: 4/20/2015

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
Aspartate aminotransferase	Dimension® Xpand Chemistry Analyzer	SGOT

Synonyms/Abbreviations
AST, SGOT

Department
Chemistry

Form revised 2/02/2007

2. ANALYTICAL PRINCIPLE

The aspartate aminotransferase method is an adaptation of the methodology recommended by the International Federation of Clinical Chemistry (IFCC). The method uses the coenzyme pyridoxal-5-phosphate (P5P) to activate the apoenzyme and lactic acid dehydrogenase (LDH) to eliminate pyruvate interference.
Aspartate aminotransferase (AST) catalyzes the transamination from L-aspartate to a-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.

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

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

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. Avoid prolonged contact of the serum and plasma with separated red cells.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	Plasma (Lithium Heparin)
-Other Acceptable	Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum	1.0 mL
- Minimum	0.5 mL
Transport Container and Temperature	Collection tube or plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: (20-25°C) 3 days
	Refrigerated: (2-8°C) 7 days
	Frozen: (-20°C or colder) 1 month
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	Moderate or gross hemolysis. Reject sample and request redraw.
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
Aspartate Aminotransferase	Siemens, Flex® reagent cartridge, Cat. No. DF41A
Enzyme Diluent	Siemens Cat. No. 790035901

4.2 Reagent Preparation and Storage

Reagent	Aspartate Aminotransferase
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: 3 days for wells 1 - 6

Preparation	Hydrating, diluting and mixing are automatically performed by the instrument.

Reagent	**Enzyme Diluent**
Container	Reagent vial
Storage	Store at 2-8°C
Stability	• Un-reconstituted reagent 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

Calibrator	Enzyme Verifier
Preparation	• Remove vials from refrigerator and allow to stand at room temperature 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 gently for 30 seconds, then gently invert 10 times. • Let vials stand for 10 minutes, 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. Prior to use, invert 10 times and swirl gently.

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 at 2-8° C.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	Enzyme Verifier
Assay Range	0–1000 U/L
Suggested calibration level	See Reagent Package Insert for lot specific assigned values in U/L
Frequency	• Every new reagent cartridge lot. • Every 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 in triplicate
Assigned Coefficients	Standard sample size = 40 µL C_0 2.000 C_1 -3.537 Alternate sample size = 20 µL C_0 -2.000 C_1 -7.040
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	Open thawed controls: AST will be stable for 7 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.

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

Step	Action
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

AST Flex® reagent cartridge Cat. No. DF41A is required to perform this test.

Aspartate Aminotransferase is performed on the Dimension® clinical chemistry 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.

8.3	Specimen Testing
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:	40 µL, (20 µL)
Reagent 1 Volume:	100 µL
Reagent 2 Volume:	65 µL
Diluent Volume:	235 µL
Temperature:	37° C
Wavelength:	340 and 700 nm
Type of Measurement:	bichromatic rate

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

The instrument automatically calculates and prints the concentration of AST 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 - 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…
0 U/L	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: 0 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 8. No multiplication is necessary.
> 8,000 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.
> 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):	15 - 37 U/L	15 - 37 U/L
Pediatric:		
16 – 19 years	0 - 26	10 - 41
12 – 15 years	5 - 26	10 - 36
7 – 11 years	5 - 36	10 - 36
5 – 6 years	10 – 47	10 - 47
1 – 4 years	16 - 57	16 - 57
7 – 11 months	16 - 60	16 - 52
4 – 6 months	16 - 60	16 - 62
1 – 3 months	16 - 61	16 - 60
8 – 30 days	20 - 69	16 - 67
0–7 days	20 - 93	26 - 98

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

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.

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 5-test precision is observed at the standard sample size:

AST Activity	S.D.
40 U/L	>2.5 U/L
440 U/L	>8 U/L
830 U/L	>15 U/L

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0-1000 U/L

14.2 Precision

Material	Mean U/L	Standard Deviation (%CV)	
		Within-run	Total
Multiqual®			
Level 1	46	1.2 (2.7)	2.4 (5.2)
Level 2	190	1.6 (0.8)	3.9 (2.1)
Moni-Trol® Control			
Level 1	25	2.8 (11.5)	3.0 (12.3)
Level 2	120	2.9 (2.4)	3.7 (3.1)

14.3 Interfering Substances

Lipemia (Intralipid®) of 600 mg/dL (6.78 mmol/L) tripped a test report message; therefore the magnitude of interference could not be determined.

HIL Interference:
The AST 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	AST Activity U/L	Bias %
Hemoglobin (hemolysate)	50 mg/dL [0.031 mmol/L]	53	<10
Bilirubin (unconjugated)	20 mg/dL [342 µmol/L]	54	<10
Lipemia (Intralipid®)	200 mg/dL [2.26 mmol/L]	58	<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.

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 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, AST Flex® Reagent Cartridge DF41A

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, AST Flex® Reagent Cartridge DF41A, Siemens Healthcare Diagnostics Inc., 02/26/2016.
3. Package Insert, Enzyme Verifier DC19, Siemens Healthcare Diagnostics Inc, 12/2015.
4. Package Insert, Liquichek Unassayed Chemistry Controls, Bio-Rad Laboratories, 04/2016.
5. Package Insert, Enzyme Diluent, Siemens Healthcare Diagnostics Inc., 10/2012.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP C057.000		
000	3/9/12		Update owner	L Barrett	J Buss
000	3/9/12	3.2	Update specimen stability	A Chini	J Buss
000	3/9/12	5.3	Changed statement on Sug. Cal. Level	A Chini	J Buss
000	3/9/12	5.5	Correct second entry of 'and' to 'or'	L Barrett	J Buss
000	3/9/12	6.2	Add note for local practice open dating	L Barrett	J Buss
000	3/9/12	6.7	Add use of TEA for lot to lot runs, remove testing new calibrator lots as unknowns prior to use	L Barrett	J Buss
000	3/9/12	10.2	Corrected rounding to whole number	A Chini	J Buss
000	3/9/12	10.5	Corrected repeat criteria and dilution process, remove code QNSR	A Chini	J Buss
000	3/9/12	11.2	Title change to local terminology	L Barrett	J Buss
000	3/9/12	15	Update to standard wording	L Barrett	J Buss
000	3/9/12	16	Add current package insert	L Barrett	J Buss
000	3/9/12	17	Update reference dates	A Chini	J Buss

Version	Date	Section	Reason	Reviser	Approval
000	3/9/12	19	Remove package insert	L Barrett	J Buss
001	4/20/15		Update owner	L Barrett	R SanLuis
001	4/20/15	1,7.1	Add analyzer name	L Barrett	R SanLuis
001	4/20/15	3.2	Specify anticoagulant	L Barrett	R SanLuis
001	4/20/15	4	Add Enzyme Diluent to reagents	A Chini	R SanLuis
001	4/20/15	6	Add Unity Real Time	A Chini	R SanLuis
001	4/20/15	8.2	Remove Lynx, specify Xpand process	L Barrett	R SanLuis
001	4/20/15	10.4,10.5	Update CRR lower level (from 6 to 0)	A Chini	R SanLuis
001	4/20/15	16	Update SOP titles	L Barrett	R SanLuis
001	4/20/15	17	Add Enzyme Diluent	A Chini	R SanLuis
001	4/20/15	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13	L Barrett	R SanLuis
2	8/30/17	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
2	8/30/17	5.3	Remove specific calibration steps and reference separate SOP	L Barrett	R SanLuis
2	8/30/17	10.5	Move patient review from section 6	L Barrett	R SanLuis
2	8/30/17	10.6	Remove repeat value below AMR/CRR	L Barrett	R SanLuis
2	8/30/17	15	Update to new standard wording	L Barrett	R SanLuis
2	8/30/17	17	Update PI dates	L Barrett	R SanLuis

19. ADDENDA

None

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**
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
Alanine Aminotransferase	Dimension® Xpand Chemistry Analyzer	SGPT

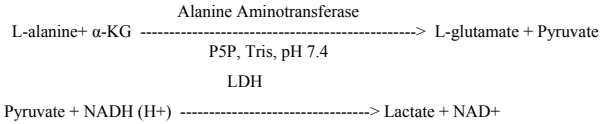
Synonyms/Abbreviations
ALT, SGPT, Included in Batteries/Packages: COMP, LIVP

Department
Chemistry

Form revised 2/02/2007

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 (Lithium 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 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.
Other Considerations	Allow Red Top or SST 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
Alanine Aminotransferase	Siemens, Flex® reagent cartridge, Cat. No. DF143
Enzyme Diluent	Siemens Cat. No. 790035901

4.2 Reagent Preparation and Storage

Reagent	**Alanine Aminotransferase**
Container	Reagent cartridge
Storage	Store at 2-8°C
Stability	• Stable until expiration date stamped on reagent cartridges. • Sealed wells on the instrument are stable for 30 days. • Open well stability o 3 days for wells 1 - 6 o 30 days for wells 7 - 8
Preparation	Hydration, mixing and diluting are automatically performed by the instrument.

Reagent	**Enzyme Diluent**
Container	Reagent vial
Storage	Store at 2-8°C

Stability	• Un-reconstituted reagent 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
ENZ II CAL	Siemens Dimension®, Cat. No. DC143

5.2 Calibrator Preparation and Storage

Calibrator	ENZ II CAL
Preparation	Calibrator is ready for use. No preparation is required.
Storage/Stability	• Store at 2-8°C • **Unopened calibrator:** stable until expiration date on 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	• 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
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	Open thawed control: ALT 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.

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
- 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.

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

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

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 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…
< 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:		
16 – 19 years	19 - 49	24 - 54
14 – 15 years	19 - 44	24 - 59
12 – 13 years	24 - 44	24 - 68
10 – 11 years	24 - 44	24 - 49
4 – 9 years	24 - 49	24 - 49
1 – 3 years	24 - 59	19 - 59
7 – 11 months	26 - 55	26 - 59
4 – 6 months	26 - 51	26 - 55
1 – 3 months	26 - 61	27 - 54
8 days – 30 days	22 - 46	24 - 54
0 – 7 days	21 - 54	20 - 54

11.2 Critical Values

None established

11.3 Standard Required Messages

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 80 mg/dL	68 140	<10
Bilirubin (conjugated)	30 mg/dL 40 mg/dL	71 144	<10
Lipemia Intralipid®	200 mg/dL 600 mg/dL	50 144	<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.

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. 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 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., 01/30/2015.
3. Package Insert, ENZ II CAL, Siemens Healthcare Diagnostics Inc., 05/2016.
4. Package Insert, Unassayed Liquichek Chemistry Controls, Bio-Rad Laboratories, 04/2016.
5. Package Insert, Enzyme Diluent, Siemens Healthcare Diagnostics Inc., 10/2012.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
000	4/20/15	3.2	Specify anticoagulant	L Barrett	R SanLuis
000	4/20/15	6.4,6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
000	4/20/15	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13	L Barrett	R SanLuis
1	8/30/17	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
1	8/30/17	5.3	Remove specific calibration steps and reference separate SOP	L Barrett	R SanLuis
1	8/30/17	10.5	Move patient review from section 6	L Barrett	R SanLuis
1	8/30/17	10.6	Remove repeat value below AMR/CRR	L Barrett	R SanLuis
1	8/30/17	15	Update to new standard wording	L Barrett	R SanLuis
1	8/30/17	17	Update PI dates	L Barrett	R SanLuis

19. ADDENDA

None