TRAINING UPDATE

Lab Location:	SGMC & WAH	**Date Distributed:**	8/12/2019
Department:	Core Lab, Phleb and Processing	**Due Date:**	8/31/2019
		Implementation:	**8/27/2019**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Ammonia by Dimension Vista® System SGAH.C865 v3

Description of change(s):

Major changes to SOP are in section 3 –

- ***additional info added sample to collection AND***
- ***new rejection criteria added***

Refer to yellow highlights in attached SOP

Section	Reason
3.1	Add fasting preferred and transportation
3.2	Add reject if >30 min from collection
10.6	Update instruction for result above CRR
17	Update PI revision dates

This revised SOP will be implemented on August 27, 2019

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

Technical SOP

Title	**Ammonia by Dimension Vista® System**
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**

Form revised 2/02/2007

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Ammonia	Dimension Vista® System	NH3

Synonyms/Abbreviations
NH3

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Dimension Vista® Ammonia (AMM) method is an enzymatic method that uses glutamate dehydrogenase (GLDH) and a stabilized NADPH analog. Ammonia reacts with α-ketoglutarate and reduced cofactor to form L-glutamate and the cofactor. The reaction is catalyzed by glutamate dehydrogenase. The decrease in absorbance due to the oxidation of the reduced cofactor is monitored at 340/700 nm and is proportional to the ammonia concentration.

$$\alpha\text{-ketoglutarate} + NH_4^+ + \text{reduced cofactor} \xrightarrow{\text{GLDH}} \text{L-glutamate} + \text{cofactor} + H_2O$$

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	A fasting specimen is preferred.
Specimen Collection and/or Timing	• A free-flowing venous (or arterial) blood sample should be collected into a specimen tube (preferably pre-chilled). • Do not collect via indwelling catheters or capillary puncture. • Do not collect after physical exercise. • Smoking should be avoided for at least 9 hours before sample collection. The tube should be completely filled and stored tightly capped on ice.
Special Collection Procedures	The sample should be transported on ice to the laboratory **immediately, separated within 20 minutes of collection and analyzed immediately**.
Other	N/A

3.2 Specimen Type & Handling

Criteria		
Type	**-Preferred**	Plasma (Lithium heparin)
	-Other Acceptable	None
Collection Container		Mint green top tube (PST)
Volume	**- Optimum**	1.0 mL
	- Minimum	0.5 mL
Transport Container and Temperature		Collection container or plastic vial on ice.
Stability & Storage Requirements	Room Temperature:	Not stable
	Refrigerated:	2 hours (separated specimens)
	Frozen:	Not stable

Criteria	
Timing Considerations	Centrifuge immediately and analyze within 30 minutes of collection.
Unacceptable Specimens & Actions to Take	Reject samples that are **received greater than 30 minutes after the collection time or are not on ice.** 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	Do not use hemolyzed samples. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)
Other Considerations	Concentrations may more than double in plasma when stored at room temperature for 6 hours.

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. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.

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 / Kits	Supplier & Catalog Number
Ammonia	Siemens, Flex® reagent cartridge, Cat. No. K3119

4.2 Reagent Preparation and Storage

Reagent	**Ammonia**
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 60 days. • Open well stability: 3 days for wells 1 - 12
Preparation	All reagents are liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
CHEM 3 CAL	Siemens Dimension Vista®, Cat. No. KC130A

5.2 Calibrator Preparation and Storage

Calibrator	CHEM 3 CAL
Preparation	Calibrator is ready for use. No preparation is required.
Storage/Stability	• Store at 2 – 8°C • **Unopened Calibrator:** until expiration date on the box. • **Opened Calibrator:** once the stopper is punctured, stable for 24 hours when stored on board the Dimension Vista System.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	CHEM 3 CAL
Assay Range	10 – 750 µmol/L
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in µmol/L
Frequency	• Every new reagent cartridge lot. • Every 60 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	Two levels

5.4 Calibration Procedure

Auto Calibration:

1. Place the required calibrator vials in a carrier. Make sure the barcode labels are entirely visible through the slots.
2. Place the carrier in the loading area.
3. Position the carrier with the labels facing away from the user.
4. Press the **Load** button.
5. Automatic calibration requires that calibrators be on the instrument. As the time for processing approaches, the instrument reviews onboard inventory for the appropriate calibrators.

Manual Calibration:

1. Verify that calibrators and reagents are in inventory on the instrument.
2. Press **System > Method Summary > Calibration**.

3. Select a method from the sidebar menu. Press the **Order Calibration** button on the screen.
4. Verify that the information on the screen is correct. Verify that the calibrator lot is correct using the drop-down menu.
   a. When calibrating using Vials press **OK**.
   b. When calibrating using Cups, check the Use Cups box. This displays the rack and cup position fields. For additional cups use the positions in ascending order. Be sure to use the number of calibration levels and cups as specified in the method IFU. Scan the rack barcode and place calibrator cups in an adapter in position 1 on a rack. Press **OK** and load the rack on the instrument.
5. The status field in the calibration screen changes sequentially to Awaiting Scheduling, Preparing Calibrators and Processing.

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™ Ethanol/Ammonia Control Levels 1, 2 and 3	Bio-Rad Laboratories Cat. No. 271, 272 and 273

6.2 Control Preparation and Storage

Control	Liquicheck Ethanol/Ammonia Controls
Preparation	Before loading vials onto the instrument, gently swirl the contents to ensure homogeneity.
Storage/Stability	**Unopened**: Stable until the expiration date at 2-8°C. **Opened**: Stable for 20 days at 2-8°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 Vista® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension Vista® 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 Vista® 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

- Aliquot Plates
- System Fluids
- Assorted calibrated pipettes (MLA or equivalent) and disposable tips

8. PROCEDURE

AMM Flex® reagent cartridge Cat. No. K3119 is required to perform this test.

Ammonia is performed on the Dimension Vista® 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	Sample Processing
1.	A sample rack holding tubes or cups is placed on the rack input lane.
2.	The sample shuttle moves the rack to the barcode reader which identifies the rack and samples to the system.
3.	The rack moves into the sample server and to the rack positioner.
4.	At the same time, aliquot plates move from the aliquot loader into position.
5.	The aliquot probe aspirates the sample from the tubes or cups and dispenses it into the wells of the aliquot plates.
6.	After each aspirate-dispense action, the probe is thoroughly rinsed inside and out to prevent sample carryover.
7.	When sample aspiration is completed, the sample server moves the rack back to the sample shuttle, where it is placed on the output lane and can be removed by the operator.

8.2	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension Vista® QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension Vista® Operator's 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 Vista® 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:	20 µL
Reagent Volume:	130 µL
Reaction Time:	5.9 minutes
Test Temperature:	37°C
Wavelength:	340 & 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 Ammonia in µmol/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

µmol/L

10.4 Clinically Reportable Range (CRR)

10 – 2,250 µmol/L

10.5 Review Patient Data

Each result is reviewed for error messages. Refer to the Dimension Vista system manual "Error messages" section for troubleshooting. Resolve any problems noted before issuing patient reports.

10.6 Repeat Criteria and Resulting

IF the result is …	THEN…
< 10 µmol/L	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 10 µmol/L
≥ 750 µmol/L	**On Board Automated Dilution:** Results ≥ 750 µmol/L will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
1,500 µmol/L	**Manual Dilution:** Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 3 **Diluent:** reagent grade water Enter dilution factor as a whole number on the "Enter Sample Data" screen.

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IF the result is …	THEN…
> 2,250 µmol/L	If the recommended dilution does not give results within the clinically reportable range, report as: "> 2,250 µmol/L -REP" Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of results.

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

11. EXPECTED VALUES

11.1 Reference Ranges

11 – 32 µmol/L

11.2 Critical Values

>199 µmol/L

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

The major source of circulating ammonia is the gastrointestinal (GI) tract. Under normal conditions, ammonia is metabolized to urea by liver enzymes. Several diseases, both inherited and acquired, cause elevated ammonia (hyperammonemia). The inherited deficiencies of urea cycle enzymes are the major cause of hyperammonemia in infants. Acquired hyperammonemia most often results from liver disease, renal failure, and Reye's syndrome. Elevated ammonia is toxic to the central nervous system.

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 Vista Operator's Guide.

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

AMM Concentration	Acceptable S.D. Maximum
41 µmol/L	6.9 µmol/L
206 µmol/L	25.9 µmol/L

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

10 – 750 µmol/L

14.2 Precision

Material	Mean µmol/L	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Liquichek Ethanol Ammonia			
Level 1	26	1.6	1.9
Level 2	109	1.4	1.9
Level 3	331	2.1	2.9

14.3 Interfering Substances

Dextran 40 at 1500 mg/dL increases AMM results by 35% at an ammonia concentration of 50 µmol/L and increases AMM results by < 10% at an ammonia concentration of 250 µmol/L.
Dextran 40 at 250 mg/dL increases AMM results by 10% at an ammonia concentration of 50 µmol/L.
Immunoglobulin G (IgG) at 5 g/dL increases AMM results by 30% at an ammonia concentration 50 µmol/L and increases AMM results by < 10% at an ammonia concentration of 250 µmol/L.
Triglycerides at 3000 mg/dL tripped a test report message; therefore the magnitude of the interference could not be determined.

HIL Interference:

The AMM method was evaluated for interference according to CLSI/NCCLS EP7-A2.8 Bias is the difference in the results between the control sample (without the interferent) and the test sample (contains the interferent) expressed in percent. Bias exceeding 10% is considered interference.

Substance tested	Substance Concentration	AMM µmol/L	Bias %
Hemoglobin (hemolysate)	75 mg/dL 500 mg/dL	50 250	12 17
Bilirubin (unconjugated)	80 mg/dL	50, 250	<10
Bilirubin (conjugated)	60 mg/dL 80 mg/dL	50 250	-16 <10
Lipemia Intralipid®	50 mg/dL	50, 250	13, <10

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14.4 Clinical Sensitivity/Specificity/Predictive Values

None

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.

CHEM 3 CAL and Liquichek Ethanol/Ammonia Controls may cause an allergic skin reaction.
Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention

16. RELATED DOCUMENTS

1. Dimension Vista® Clinical Chemistry System Operator's Manual
2. Dimension Vista® Calibration/Verification Procedure
3. Dimension Vista® Cal Accept Guidelines
4. Dimension Vista® Calibration summary
5. Dimension Vista® Sample Processing, Startup and Maintenance procedure
6. Laboratory Quality Control Program
7. QC Schedule for Siemens Dimension Vista®
8. Laboratory Safety Manual
9. Safety Data Sheets (SDS)
10. Dimension Vista® Limits Chart (AG.F200)
11. Quest Diagnostics Records Management Procedure
12. Dimension Vista® System Error Messages Chart
13. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
14. Specimen Acceptability Requirements (Lab policy)
15. Repeat Testing Requirement (Lab policy)
16. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
18. Current package insert AMM Flex® Reagent Cartridge K3119

17. REFERENCES

1. Package Insert, AMM Flex® Reagent Cartridge K3119, Siemens Healthcare Diagnostics Inc., 06/14/2019.
2. Package Insert, CHEM 3 CAL, Siemens Healthcare Diagnostics Inc., 3/2015.
3. Package Insert, Liquichek Ethanol/Ammonia Controls, Bio-Rad Laboratories, 8/2018

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
0	1/19/17	Header	Add WAH	L Barrett	R SanLuis
0	1/19/17	3.2	Remove specimen onboard stability	L Barrett	R SanLuis
0	1/19/17	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
0	1/19/17	6.1, 6.2	Update QC material and storage	L Barrett	R SanLuis
0	1/19/17	6.4, 6.5	Replace LIS with Unity Real Time	L Barrett	R SanLuis
0	1/19/17	10.5	Move patient review from section 6	L Barrett	R SanLuis
0	1/19/17	11.2	Reformat value to eliminate ≥ sign	L Barrett	R SanLuis
0	1/19/17	15	Update to new standard wording, add warning	L Barrett	R SanLuis
0	1/19/17	17	Update QC product	L Barrett	R SanLuis
1	1/25/19	Header	Update parent facility	L Barrett	R SanLuis
1	1/25/19	3.2	Change centrifuge to within 30 min	L Barrett	R SanLuis
1	1/25/19	16	Update policy title	L Barrett	R SanLuis
1	1/25/19	17	Update PI revision dates	L Barrett	R SanLuis
2	8/2/19	3.1	Add fasting preferred and transportation	L Barrett	R SanLuis
2	8/2/19	3.2	Add reject if >30 min from collection	L Barrett	R SanLuis
2	8/2/19	10.6	Update instruction for result above CRR	L Barrett	R SanLuis
2	8/2/19	17	Update PI revision dates	L Barrett	R SanLuis

19. ADDENDA

None