DXC (AMM) AMMONIA

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☒ St. Francis Hospital Federal Way, WA ☒ St. Anthony Hospital Gig Harbor, WA ☐ Highline Medical Center Burien, WA ☐ PSC

PURPOSE

To provide instructions for the quantitative determination of ammonia on the DXC 600/800.

PRINCIPLE

AMM reagent, when used in conjunction with UniCel® DxC 600/800 System(s) and SYNCHRON® Systems Ammonia Calibrators, is intended for the quantitative determination of Ammonia concentration in human plasma.

BACKGROUND

Clinical Significance

Circulatory ammonia level in normal individuals is relatively low despite the fact that ammonia is continuously produced from dietary and amino acid metabolism. Monitoring blood ammonia levels can be useful in the diagnosis of hepatic encephalopathy and hepatic coma in the terminal stages of liver cirrhosis, hepatic failure, acute and subacute necrosis, and Reye`s syndrome. Hyperammonemia in infants may be an indicator of inherited deficiencies of the urea cycle metabolic pathway.

Methodology

AMM reagent is used to measure Ammonia by a timed endpoint method. In the assay reaction, glutamate dehydrogenase (GLDH) catalyzes the condensation of AMM and α-ketoglutarate to glutamate with the concomitant oxidation of reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) to β-nicotinamide adenine dinucleotide phosphate ($NADP^+$). The amount of NADPH oxidized is directly proportional to the amount of analyte in the sample.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 6 parts reagent. The system monitors the change in absorbance at 340 nanometers. This change in absorbance is directly proportional to the concentration of Ammonia in the sample and is used by the SYNCHRON® System(s) to calculate and express the Ammonia concentration.

RELATED DOCUMENTS

R-PO-CH0810	Quality Control Program General Laboratory
R-PO-CH0809	Quality Control Westgard Rules Statistics
R-PR-AD0540	Specimen Rejection/Cancellation Protocol
J-F-CH0820	DXC 800 Controls

M-F-CH0820	Chemistry Controls
J-F-CH0826	DXC 800 Calibrators
M-F-CH0826	Chemistry Calibrators
M-F-CH1940	DXC 600 (AMR) Analytical Measurement Range
J-F-CH1940	DXC 800 (AMR) Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn plasma is the preferred specimen. Acceptable anticoagulants are listed in PROCEDURAL NOTES section of this chemistry information sheet. Whole blood, serum and urine are not recommended for use as a sample.

Specimen Storage and Stability

Tubes should be filled completely, mixed gently by inversion, placed on ice, centrifuged immediately for 10 minutes at an RCF of 1500G and analyzed within 2 hours. Samples should not be frozen. The tubes should be tightly stoppered at all times. Centrifuge RPM`s can be calculated from the g value using the following equation:

$$RPM = \sqrt{\frac{RCF}{(0.0000118)\, r}}$$

r = Rotating radius (centimeters)
RCF = Relative centrifugal force (gravities)

Sample Type	Volume	Sample stability
Plasma	0.5mL	• Freshly drawn (Lithium Heparin or EDTA plasma), mixed gently by inversion and placed on ice • Centrifuge immediately, within 20 minutes, in cold centrifuge. Values increase rapidly at RT or refrigerated. • On Ice 2 hours • Do not freeze.

Criteria for Unacceptable Specimens

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens. See also Specimen Rejection Protocol.

REAGENTS

Contents

Each kit contains the following items:
Two Ammonia Reagent cartridges (2 x 25 tests) Kit #439770
One bottle Ammonia Calibrator Level 1 (25 µmol/L) (liquid, 5 mL)
One bottle Ammonia Calibrator Level 2 (300 µmol/L) (liquid, 5 mL)

Volumes Per Test	
Sample Volume	40uL
Total Reagent Volume	226 uL
Cartridge, Volumes	A 180uL, B 40uL and C 6uL

Reagent Constituents

Reactive Ingredients	
α-Ketoglutarate	3.23 mmol/L
ADP	1.9 mmol/L
NADPH	0.22 mmol/L
GLDH(Beef Liver)	>10 U/l

Calibrator Constituents

Ammonium Sulfate in 0.01M Sulfuric Acid	154 µmol/L

Reagent Preparation

No preparation is required.

Acceptable Reagent Performance

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your acceptance criteria.

Reagent Storage and Stability

AMM reagent, when stored unopened at +2°C to +8°C, will remain stable until the expiration date printed on the label. Once opened, the reagent is stable for 30 days at +2°C to +8°C unless the expiration date is exceeded.

CALIBRATION

Calibrator Required

SYNCHRON® Systems Ammonia Calibrators (included in the SYNCHRON Systems Ammonia Reagent kit)

Calibrator Preparation

If unopened, the SYNCHRON® Systems Ammonia Calibrators may be stored at +2°C to +8°C until the expiration date printed on the calibrator bottle. Once opened, the calibrators are stable for 60 days at +2°C to +8°C. Do not use past manufacturer's expiration date.

Calibrator Information

1. The system must have a valid calibration factor in memory before control or patient samples can be run.

2. Under typical operating conditions the AMM reagent cartridge must be calibrated every 5 days and also with certain parts replacement or maintenance procedures, as defined in the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

3. For detailed calibration instructions, refer to the UniCel DxC 600/800 System *Instructions for Use* (IFU) manual.

4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the UniCel DxC 600/800 System *Instructions for Use* (IFU) manual.

Calibrator Assigned Values

SYNCHRON Ammonia Calibrator Levels 1 and 2 are standards with ammonium sulfate weighed in, to ammonia concentrations of 25 µmol/L and 300 µmol/L, respectively.

Calibrator Summary

SYNCHRON® Systems Ammonia Calibrators are derived from human serum that has been processed and spiked with ammonium sulfate. Assay of calibrators provides a response value for the calculation of slope and offset that is utilized by the SYNCHRON System to establish a calibration curve for the reagent lot.

Calibrator Limitations

SYNCHRON® Systems Ammonia Calibrators should be used only in conjunction with SYNCHRON Systems and SYNCHRON AMM reagents. Adverse storage conditions of SYNCHRON® Systems Ammonia Calibrators may cause erroneous test results.

Traceability

Ammonia measured (analyte) in this calibrator is traceable to the manufacturer's selected measuring method. The traceability process is based on prEN ISO 17511.
Ammonia set point values were established based upon the gravimetric addition of specific quantities of the measured to achieve the appropriate concentration.
The values were verified using representative samples from this lot of calibrator and are specific to the assay methodologies of the SYNCHRON® System(s). Values determined by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

QUALITY CONTROL

See Related Documents J-F-CH0820 DXC 800 Controls & M-F-CH0820 Chemistry Controls

TESTING PROCEDURE(S)

1. If necessary, load the reagent onto the system.

2. After reagent load is completed, calibration may be required.

3. Program controls for analysis.
4. After loading controls onto the system, follow the protocols for system operation. To load samples manually refer to the FHS DXC Series Manual Sample Programming procedure. For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

CALCULATIONS

SYNCHRON® System(s) perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

PROCEDURAL NOTES

Anticoagulant Test Results

Anticoagulant	Level of anticoagulant tested
Sodium Heparin	14 Units/mL
EDTA	1.5 mg/mL

Limitations

1. Atmospheric ammonia may cause falsely elevated results.

2. Smoking is a source of ammonia contamination.

3. The presence of ammonium ions in anticoagulants may produce falsely elevated results.

4. This procedure was not validated on neonatal samples by this laboratory.

INTERFERENCES

1. The following substances were tested for interference with this methodology:

Substance	Source	Level tested	Observed effect
Bilirubin	Bovine	24 mg/dL INDEX of 20	No Significant interference (within +/- 0.4 mg/dL or 10%)
Hemolysis		INDEX of 1	Needs recollection
Lipemia		INDEX of 2	Affected. Ultracentrifugation not recommended due to warming of sample.

PERFORMANCE CHARACTERISTICS

Reference range*[3,7]

Age From	Age To	Reference Range
0 Minutes	1 Day	64-107 umol/L*
1 Day	2 Weeks	56-92 umol/L*
2 Weeks	1 Month	21-50 umol/L*
1 Month	150 Years	11-35 umol/L

*Neonatal reference ranges not established by this laboratory.

Analytic Range

The SYNCHRON® System(s) method for the determination of this analyte provides the following analytical range:

Analytical Range / reportable range

Sample type	S.I. Units
Plasma	9– 1000 µmol/L

The low end of the analytical range represents the minimum level of detection.

Reporting results outside of analytical range*

Lower limit of detection	9 umol/L	Results below 9 report < 9 umol/L
Upper limit of detection	1000 umol/L	**DO NOT DILUTE, report as >1000**

ADDITIONAL INFORMATION

For more detailed information on UniCel DxC Systems, refer to the appropriate system manual.

REFERENCES

1. Ratliff, C. R., Hall, F. F., "Blood Ammonia Returns to the Laboratory", *Laboratory Management*, 16 (1979).
2. Ratliff, C. R., Hall, F. F., "Ammonia in Plasma Enzymatic Procedure", *Selected Methods of Clinical Chemistry*, 9:85 90 (1982).
3. Tietz, Allan H.B., “Clinical Guide to Labortory Tests”, Fourth Edition, Saunders Elsevier, St. Louis, MO (2006).
4. Tietz, N. W., "Specimen Collection and Processing; Sources of Biological Variation", *Textbook of Clinical Chemistry*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1994).
5. CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, U.S. Government Printing Office, Washington, D.C. (1984).
6. Henry, J. B., *Clinical Diagnosis and Management by Laboratory Methods*, 18th Edition, W. B. Saunders Company, Philadelphia, PA (1991).
7. Tietz, N. W., ed., *Fundamentals of Clinical Chemistry*, 5th Edition, W. B. Saunders, Philadelphia, PA (2001).
8. National Committee for Clinical Laboratory Standards, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory*, Approved Guideline, NCCLS publication C28-A, Villanova, PA (1995).
9. Tietz, N. W., *Textbook of Clinical Chemistry*, 2nd Edition, W. B. Saunders Company, Philadelphia, PA (1994).
10. Young, D. S., *Effects of Drugs on Clinical Laboratory Tests*, 4th Edition, AACC Press, Washington, D. C. (1995).
11. Friedman, R. B., Young, D. S., *Effects of Disease on Clinical Laboratory Tests*, 3rd Edition, AACC Press, Washington, D.C. (1997).
12. Young, D. S., *Effects of Preanalytical Variables on Clinical Laboratory Tests*, 2nd Edition, AACC Press, Washington, D. C. (1997).
13. National Committee for Clinical Laboratory Standards, *Method Comparison and Bias Estimation Using Patient Samples*, Approved Guideline, NCCLS publication EP9-A, Villanova, PA (1995).
14. National Committee for Clinical Laboratory Standards, *Preliminary Evaluation of Quantitative Clinical Laboratory Method*, Approved Guideline, NCCLS publication EP10-A, Wayne, PA (1998).
15. Beckman Coulter Ireland Inc., Mervue Business Park, Mervue, Galway, Ireland (353 91 774068) Beckman Coulter, Inc., 4300 N. Harbor Blvd., Fullerton, CA 92835

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