BECKMAN COULTER

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SYNCHRON® System(s) Chemistry Information Sheet

AMM Ammonia

REF 439770

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

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

CLINICAL SIGNIFICANCE

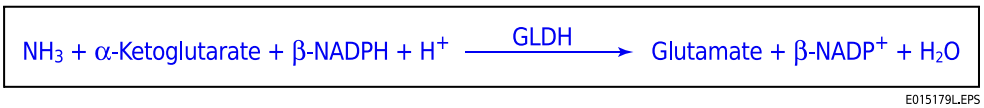
Circulatory ammonia level in normal individuals is relatively low despite the fact that ammonia is continuously produced from dietary and amino acid metabolism. Monitoring blood ammonia levels can be useful in the diagnosis of hepatic encephalopathy and hepatic coma in the terminal stages of liver cirrhosis, 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.[1,2]

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.

CHEMICAL REACTION SCHEME



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SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.[3] Freshly drawn plasma is the preferred specimen. Acceptable anticoagulants are listed in PROCEDURAL NOTES section of this chemistry information sheet. Whole blood, serum or 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 30 minutes. 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:

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

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

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE VOLUME

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

Two Ammonia Reagent cartridges (2 x 25 tests)
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	40 µL
Total Reagent Volume	226 µL
Cartridge Volumes	
A	180 µL
B	40 µL
C	6 µL

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

α-Ketoglutarate	3.23 mmol/L
ADP	1.9 mmol/L
NADPH	0.22 mmol/L
GLDH (Beef liver)	>10 U/L

Also non-reactive chemicals necessary for optimal system performance.

CALIBRATOR CONSTITUENTS

Ammonium Sulfate in 0.01M Sulfuric Acid	154 µmol/L

Also non-reactive chemicals necessary for optimal system performance.

Avoid skin contact with reagent. Use water to wash reagent from skin.

EUROPEAN HAZARD CLASSIFICATION

Ammonia Reagent (Compartment B)	Xn;R22	Harmful if swallowed.
	S37/39	Wear suitable gloves and eye/face protection.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

At least two levels of control material
Ammonia-free deionized water

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 facility's 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.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

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

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

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 unless the expiration date is exceeded.

⚠ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.[4]

Calibrator storage location:

CALIBRATION 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 SYNCHRON LX *Maintenance Manual and Instrument Log*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.
3. For detailed calibration instructions, refer to the SYNCHRON LX *Operations Manual*, or 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 SYNCHRON LX *Diagnostics and Troubleshooting Manual*, or 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 measurand (analyte) in this calibrator is traceable to the manufacturer's selected measuring method.[2] 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 measurand 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

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

Table 1.0 Quality Control Material

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

1. If necessary, load the reagent onto the system.
2. After reagent load is completed, calibration may be required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations.

For detailed testing procedures, refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

CALCULATIONS

The SYNCHRON® System(s) performs 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.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

Each laboratory should establish its own reference intervals based upon its patient population. The reference intervals listed below were taken from literature and a study performed on SYNCHRON Systems.[5]

Table 2.0 Reference intervals

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Literature	Plasma	19 – 60 µg/dL	11 – 35 µmol/L
SYNCHRON	Plasma	16 – 60 µg/dL	9 – 35 µmol/L

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Laboratory			

Refer to References (5,6,7) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

ANTICOAGULANT TEST RESULTS

The following anticoagulants were assessed by Deming regression analysis with a minimum of 50 paired lithium heparin plasma and other plasma samples. Ammonia values of lithium heparin plasma (X) ranging from 10 to 936 µmol/L were compared with the ammonia values of other plasma (Y) yielding the following results.

Table 3.0 Anticoagulant Test Results

ANTICOAGULANT	LEVEL OF ANTICOAGULANT TESTED	DEMING REGRESSION ANALYSIS
Sodium Heparin	14 Units/mL	Y = 1.002X - 1.0; r = 0.9995
EDTA	1.5 mg/mL	Y = 1.112X - 5.1; r = 0.9992

LIMITATIONS

1. Atmospheric ammonia may cause falsely elevated results.
2. Smoking is a source of ammonia contamination.[8]
3. The presence of ammonium ions in anticoagulants may produce falsely elevated results.
4. This procedure is not validated on neonatal samples.
5. Sample results which are below the analytical range lower limit of 9 µmol/L (16 µg/dL) should be reported as "< 9.0 µmol/L" ("< 16 µg/dL").

6. Sample results greater than 1000 µmol/L (1700 µg/dL) should be diluted with ammonia-free deionized water and reanalyzed.

INTERFERENCES

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

Table 4.0 Interferences

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT
Bilirubin	Bovine	24 mg/dL	NSI[a]

a NSI = No significant interference (within ± 10.0 µmol/L or 4%).

2. Use of hemolyzed samples is not recommended because lysed red blood cells may elevate ammonia concentration in the sample.
3. Lipemic samples greater than 1+ (visual turbidity) should be ultracentrifuged and the analysis performed on the infranate.
4. Refer to References (9,10,11) for other interferences caused by drugs, disease and preanalytical variables.

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

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

Table 5.0 Analytical Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Plasma	16 – 1700 µg/dL	9 – 1000 µmol/L

The low end of the analytical range represents the minimum level of detection. Sample values greater than 1000 µmol/L (1700 µg/dL) should be diluted with ammonia-free deionized water and reanalyzed. Sample results which are below the analytical range limit of 9 µmol/L (16 µg/dL) should be reported as "< 9.0 µmol/L".

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 6.0 Reportable Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for AMM determination is 9 µmol/L (16 µg/dL).

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of plasma samples to an accepted clinical method.

Plasma (in the range of 15 to 832 µmol/L):

Y (SYNCHRON LX Systems)	= 1.014X - 0.7
N	= 56
MEAN (SYNCHRON LX Systems)	= 129
MEAN (SYNCHRON CX Systems)	= 128
CORRELATION COEFFICIENT (r)	= 0.9985

Refer to References (12) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON® System(s) should exhibit imprecision values less than or equal to the maximum performance limits listed below. Maximum performance limits were derived by an examination of the precision of various methods, proficiency test summaries, and literature sources.

Table 7.0 Maximum Performance Limits

TYPE OF PRECISION	SAMPLE TYPE	Maximum Performance Limits		CHANGEOVER VALUE[a]		% CV
		SD (µmol/L)	SD (µg/dL)	MEAN (µmol/L)	MEAN (µg/dL)	
Within-run	Plasma	5.0	8.5	250.0	425.0	2.0
Total	Plasma	7.5	12.8	250.0	425.0	3.0

a When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Comparative performance data for the SYNCHRON LX System evaluated using the NCCLS Guideline EP10-A appears in the table below.[13] Each laboratory should characterize their own instrument performance for comparison purposes.

Table 8.0 NCCLS EP10-A Precision Estimate Method

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points[a]	Test Mean Value (µmol/L)	EP10-A Calculated Means & Point Estimates	
						SD	%CV
Within-run	Plasma	Control 1	1	15	47	1.6	3.4
	Plasma	Control 2	1	15	130	1.5	1.2
	Plasma	Control 3	1	15	621	2.6	0.4
Total	Plasma	Control 1	1	15	47	2.5	5.4
	Plasma	Control 2	1	15	130	2.1	1.6
	Plasma	Control 3	1	15	621	5.0	0.8

a The point estimate is based on the pooled data from one system, run for five days, one run per day, three observations per run, on an instrument operated and maintained according to the manufacturer's instructions.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX® System and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

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

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REFERENCES

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4. CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, U.S. Government Printing Office, Washington, D.C. (1984).
5. Henry, J. B., *Clinical Diagnosis and Management by Laboratory Methods*, 18th Edition, W. B. Saunders Company, Philadelphia, PA (1991).
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9. Young, D. S., *Effects of Drugs on Clinical Laboratory Tests*, 4th Edition, AACC Press, Washington, D. C. (1995).
10. Friedman, R. B., Young, D. S.,*Effects of Disease on Clinical Laboratory Tests*, 3rd Edition, AACC Press, Washington, D.C. (1997).
11. Young, D. S., *Effects of Preanalytical Variables on Clinical Laboratory Tests*, 2nd Edition, AACC Press, Washington, D. C. (1997).
12. National Committee for Clinical Laboratory Standards, *Method Comparison and Bias Estimation Using Patient Samples*, Approved Guideline, NCCLS publication EP9-A, Villanova, PA (1995).
13. National Committee for Clinical Laboratory Standards, *Preliminary Evaluation of Quantitative Clinical Laboratory Method*, Approved Guideline, NCCLS publication EP10-A, Wayne, PA (1998).

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