Ammonia (AMM) - Plasma
Beckman UniCel DxC Systems

Technical Procedure 3104

For *In Vitro* Diagnostic Use Only

Principle

Intended Use

AMM reagent, when used in conjunction with UniCel® DxC 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

$$NH_3 + \alpha\text{-Ketoglutarate} + \beta\text{-NADPH} + H^+ \xrightarrow{\text{GLDH}} \text{Glutamate} + \beta\text{-}NADP^+ + H_2O$$

GLDH= glutamate dehydrogenase
NADPH= nicotinamide adenine dinucleotide phosphate

Specimen

Acceptable Sample Containers

13 x 75 Purple Top BD tubes (EDTA)
Purple Top BD microtainers (EDTA)

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 and urine are not recommended for use as a sample.

Specimen Processing, Storage and Stability

Tubes should be filled completely, mixed gently by inversion and placed on ice immediately after collection.

For in-patients, deliver sample to the laboratory within 30 minutes. Centrifuge immediately for 3 minutes at an RCF of 4400G or 4 minutes at an RCF of 2100G. Remove tube from centrifuge immediately after centrifugation, separate plasma from cells and analyze within one hour after sample was collected.

For outpatients, centrifuge immediately for 10 minutes at an RCF of 1200G. Remove tube from centrifuge immediately after centrifugation and separate plasma from cells. Refrigerate plasma at +2°C to +8°C if the sample will arrive at the laboratory within 2 hours. If delivery time will be longer than 2 hours, freeze immediately with dry ice. Place sample in 2 sample bags and place it next to dry ice in styrofoam holding container. Sample will freeze within 20 min-

utes. Courier will transfer sample to a transport container containing dry ice. Frozen samples will be stable for up to 6 hours if frozen at -20°C or colder.

The refrigerated and frozen stability times were validated in an in-house study.
For the refrigerated samples, ammonia was initially run, the samples were refrigerated for 2 hours and then re-analyzed.
Plasma (in the range of 15 to 306 µmol/L):

Y (2 hr refrigerated sample) Deming slope & intercept	= 1.068X - 0.175
N	= 99
Mean (2 hr refrigerated sample)	= 45
Mean (Initial sample run)	= 42
Correlation Coefficient (r)	= 0.983

For the frozen samples, ammonia was initially run, the samples were frozen at -20°C for 6 hours, thawed and then re-analyzed.
Plasma (in the range of 13 to 187 µmol/L):

Y (6 hr frozen sample) Deming slope & intercept	= 1.017X + 1.75
N	= 123
Mean (6 hr frozen sample)	= 51
Mean (Initial sample run)	= 49
Correlation Coefficient (r)	= 0.975

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*.

Criteria for Unacceptable Specimens

Refer to the *Procedural Notes* section of this chemistry information sheet for information on unacceptable specimens.

Reagents

Contents

Each kit contains the following items: **Kit Reorder # 439770**
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

Testing Procedure

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 as directed in the as defined in the UniCel DxC 800 Systems *Instructions for Use* (IFU) manual.
4. After loading samples and controls onto the system, follow the protocols for system operation as directed in the UniCel DxC 800 Systems *Instructions for Use* (IFU) manual.

Calculations

UniCel DxC Systems 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.

If the dilution was programmed in Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol.

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

The following reference intervals were taken from literature and a study performed on SYNCHRON Systems.(5)

Reference Intervals

Intervals	Sample Type	Conventional Units	S.I. Units	
Literature	EDTA Plasma	19 - 60 µg/dL	11 - 35 µmol/L	
SYNCHRON	EDTA Plasma	16 - 60 µg/dL	9 - 35 µmol/L	
UCDMC	EDTA Plasma	94 - 153 µg/dL	0 to 14 days	55 - 90 µmol/L
	EDTA Plasma	49 - 99 µg/dL	14 days to 2 yrs	29 - 58 µmol/L
	EDTA Plasma	< 16 - 60 µg/dL	> 2 yrs to adult	< 9 - 30 µmol/L

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

Pediatric reference intervals are cited from literature. No in-house studies were performed. This information should serve as a general guideline.

Critical Values

An ammonia result > 250 µmol/L is considered a critical value for all patients < 18 years old and should be called immediately to the attending physician or charge nurse. Critical ammonia results for each patient are to be called once per 24 hours.

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.

Acceptable Anticoagulants

Anticoagulant	Level Tested 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