AMMONIA

OSR61154 | **2 x 16 mL Reagent
1 x 3 mL Standard** | **R1**

Intended Use
Reagent for the quantitative determination of Ammonia (NH_3) concentrations in human plasma on the Beckman Coulter AU® chemistry analyzers.

Summary[1,2,3]
Ammonia, derived from the catabolism of amino acids and from the action of intestinal bacteria on dietary protein, is converted to urea in the liver hepatocytes and so rendered non toxic. Under normal circumstances the concentration of ammonia in the circulation remains low, typically less than 50 µmol/L (85 µg/dL). Studies have shown that excess ammonia can have a toxic effect on the central nervous system and clinical manifestations are typically neurological disturbances.
Elevated levels of ammonia may be either due to: (i) Inborn errors of metabolism; or (ii) Secondary to other conditions.
Inborn errors of metabolism are the major cause of elevated ammonia in infants and usually the result of urea cycle enzyme deficiencies. Inherited disorders affecting the metabolism of the dibasic amino acids (lysine and ornithine) and those involving the metabolism of organic acids may also produce elevated levels of circulating ammonia. Elevated ammonia may also be observed in severe liver failure as may occur in Reye's Syndrome, viral hepatitis or cirrhosis.

Methodology[1]
A number of methods have been developed for the estimation of plasma ammonia and these can be broadly classified into either indirect or direct methods. In the indirect procedures, ammonia is first of all isolated, for example by the addition of alkali or the use of a cation exchange resin, after which it is measured colourimetrically by nesslerization or Berthelot reaction. These procedures are not easily automated or require dedicated equipment. Direct procedures, such as enzymatic methods, are more widely used in routine laboratories as they do not require the separation of ammonia from the specimen prior to the analytical step. Direct procedures are therefore more easily automated. The Beckman Coulter ammonia reagent is a direct enzymatic procedure based on the following reaction sequence:-

$$NH_4 + \alpha\text{-ketoglutarate} + NADH \xrightarrow{\textit{Glutamate dehydrogenase (GLDH)}} \text{Glutamate} + NAD + H_2O$$

The reagent contains LDH in excess, to rapidly reduce endogenous pyruvate so that it does not interfere with the assay system. The Beckman Coulter Ammonia reagent also incorporates a patented stabilization process which renders the reagent stable in the liquid phase.

System Information

For AU400/400e/480, AU600/640/640e/680 and AU2700/5400 Beckman Coulter AU Analyzers.

Reagents
Active ingredients

Ammonia Reagent	
α-Ketoglutarate	7.5 mmol/L
NADH	>0.2 mmol/L
GLDH (Micro-organism)	>4000 U/L
LDH (Micro-organism)	>30,000 U/L
Tris Buffer	100 mmol/L
Also contains preservatives.	
pH 8.7 ± 0.1 at 20°C	
Ammonia Standard	
Ammonium sulphate	59 µmol/L (100 µg/dL)

Precautions
1. For *in vitro* diagnostic use. Do not ingest. Harmful if swallowed. Avoid contact with skin and eyes. If spilled, thoroughly wash affected areas with water.
2. Contains sodium azide. Sodium azide preservative in diagnostic reagents may react with lead joints in copper drain lines to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

Preparation of reagents
The reagents and standard are supplied ready to use.

Stability and Storage
1. The unopened reagent and standard are stable until the expiration date when stored at 2-8°C
2. Once opened the reagent and standard are stable in the bottles provided until the expiry date stated, provided that it is capped when not in use and stored at 2-8°C. When stored on board the reagent is stable for 14 days.

Indications of Reagent Deterioration
Turbidity and/or failure to recover control values within the assigned range.

Specimen Collection and Preparation[1]
Plasma, collected with EDTA or heparin (not ammonium heparin) into an evacuated collection tube is recommended. Ideally, the collection tube should be completely filled with blood and immediately placed on ice. Centrifuge (cold) the sample as soon as possible and separate plasma and store at 2-4°C until analysis.

Sample Storage and Stability
Ammonia samples are stable for 3 hours at 2-4°C or 24 hours at –20°C.[1]

Interfering substances
1. Hemolyzed samples should not be used as erythrocytes contain levels of ammonia approximately 3 times that of plasma.[1]
2. No interference from pyruvate was observed up to a level of 0.01 mg/dL (0.75 mmol/L).
3. No interference from ALT was observed up to a level of 2400 U/L.

4. Reliable estimations of ammonia can only be achieved if steps are taken to avoid contamination from ammonia. Sources of contamination include, but are not restricted to, cigarette smoking (patient and collection staff), laboratory atmosphere and laboratory glassware.
5. Young DS[4] has published a comprehensive list of drugs and substances which may interfere with this assay.

PROCEDURE

Materials Provided
Beckman Coulter Ammonia Reagent OSR61154
Beckman Coulter Ammonia Standard

Suggested Analytical Parameters
Refer to the Methodology Section located in the respective analyzer's Operator's Manual.

Calibration
The calibration frequency for this procedure is 7 days. Calibration of this ammonia procedure is accomplished by use of Beckman Coulter Ammonia standard provided in the kit. The standard has been verified against an in-house Ammonium Standard Solution that is traceable to Standard Reference Materials from the US National Institute of Standards and Technology (NIST).
Recalibration of this procedure is required when a reagent lot number has changed or there is an observed shift in control values, if a critical part of the analyzer is replaced or, if a major preventative maintenance procedure was performed on the analyzer.

Quality Control
During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Results in µmol/L will be automatically printed for each sample assayed. To work in (µg/dL), the result must be multiplied by 1.7.

Dynamic Range

Beckman Coulter ammonia procedure is linear from 10 to 600 µmol/L (17 - 1020 µg/dL).
Specimens with Ammonia concentrations greater than 600 µmol/L (1020 µg/dL) should be diluted with ammonia free water and reassayed. Multiply results by the dilution factor.

Expected Values[5]

16 - 53 µmol/L (27 - 90 µg/dL)

The quoted values were derived from a normal population and should serve as a guide only. It is recommended that each Laboratory verify this range or derives a reference interval for the population that it serves.[6]

Specific Performance Characteristics

The following data was obtained using the Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may vary.

Precision:[8]
Estimates of precision, based on CLSI recommendations[7], are less than 5% within run and total precision is less than 5%. Assays of control sera products were performed and the data produced following the CLSI guidlines above.

N= 20	WITHIN RUN		TOTAL	
Mean (µmol/L)	SD	CV%	SD	CV%
116	5.0	4.3	5.1	4.4
293	4.3	1.5	8.4	2.8

Method Comparison[8,9]
A comparison of this Beckman Coulter ammonia method (Method 1) vs Arkray ammonia method (Method 2) was run on an AU640 utilizing 44 patient samples. The resulting data is as follows:

Correlation Coefficient:	r = 0.9373
Regression equation:	Method 1 = 1.06X + 2.7
Range of patients:	17 - 122 µmol/L

Analytical Sensitivity (Lower Detection Limit)
The lowest detection limit (LDL) for this method was determined by assaying replicates of analyte free water (deionised). The mean and standard deviation were determined and LDL was calculated using the formula:

$$LDL = \overline{x} + (2 \times s)$$

Where: $\overline{x}$ = mean value of replicates
s = standard deviation of replicates (n - 1).

When run as recommended the lowest detection limit is 10 µmol/L (17 µg/dL).

References

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2. Tietz Textbook of Clinical Chemistry. Burtis CA and Ashwood ER (Eds). Second Edition, WB Saunders Company, 1994; 32:1485-88.
3. The Diagnosis of Urea Cycle Disorders, Lab Medica International, May/June 1993; 13-17.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests Third Edition 1990; 3: 30-2.
5. Ektachem Multilayer Dry Film Assay for Ammonia Evaluated. J Clin Chem 1985: Vol 31:12:2012-2014.
6. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
7. Clinical and Laboratory Standards Institute. User evaluation of Precision Performance of Clinical Chemistry Devices. CLSI/NCCLS, 1984, CLSI/NCCLS Publication EP5-T
8. Meller A. "Evaluation and Implementation of an Alternative Method for Measurement of Blood Ammonia". Department of Clinical Biochemistry, Newcastle upon Tyne Hospitals NHS Trust, 2006.
9. Data is on file for specific AU analyzers

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