REF	OSR61154
REAG 1	2 x 16 mL
STD	1 x 3 mL

Infinity™ Ammonia Reagent for Beckman Coulter AU Chemistry Analyzers

Rx ONLY

IVD

INTENDED USE

Reagent for the quantitative determination of Ammonia (NH_3) concentrations in human plasma for use 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 nontoxic. 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 Infinity™ ammonia reagent is a direct enzymatic procedure based on the following reaction sequence:

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

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.

REAGENT AND STANDARD COMPOSITION

Ammonia Reagent

α-Ketoglutarate	7.5 mmol/L
NADH	> 0.2 mmol/L
GLDH	> 4,000 U/L
LDH	> 30,000 U/L
Tris Buffer 100 mmol/L	
Preservative	

Ammonia Standard

Ammonium Chloride	59 µmol/L (100 µg/dL)

WARNING AND PRECAUTIONS

1. For in vitro diagnostic use only. Do not ingest. Harmful if swallowed. Avoid contact with skin and eyes. If spilled, thoroughly wash affected areas with water.
2. Contains sodium azide (0.1% w/v). 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. For further information consult the Safety Data Sheet.
3. This product contains animal source material. Handle and dispose of this product as if it were potentially infectious.

REAGENT PREPARATION

Reagent and Standard are provided 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 PREPARATION1

It is recommended that human plasma be collected in EDTA or heparin (not ammonium heparin). 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]

LIMITATIONS

Interfering Substances[4]

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 0.75 mmol/L.
3. No interference from ALT was observed up to a level of 4000 U/L.
4. Bilirubin: No significant interference up to 17.4 mg/dL unconjugated Bilirubin.
5. Lipemia: No significant interference up to 50 mg/dL Intralipid®.
6. 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, laboratory glassware or other reagents on the carousel which contain Ammonia. In the case of the latter, avoid use of the ammonia containing reagents together with OSR61154 to mitigate against atmospheric ammonia transfer. Contact your local Beckman Coulter representative for further information.

Dynamic Range

The 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.

Ammonia

ASSAY PROCEDURE

Materials Provided

- Infinity™ Ammonia Reagent
- Infinity™ Ammonia Standard

Suggested Analytical Parameters

Refer to the User Guide Accompanying the instrument.

Calibration

The calibration frequency for this procedure is 7 days. Calibration of this ammonia procedure is accomplished by use of the Infinity™ Ammonia Standard provided in the kit. The Standard has been manufactured gravimetrically using a material traceable to an in-house certified material.

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.

EXPECTED VALUES[5]

18 - 72 µmol/L (31 - 123 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.

SPECIFIC PERFORMANACE CHARACTERISTICS

Precision[6]

Estimates of precision, based on CLSI recommendations, are less than 5% within run and total precision is less than 5%. Two levels (42.4 and 192 µmol/L) of commercially available controls were evaluated over a period of 20 days, completing two assay runs per day and using two replicates per run (N=80 samples).

N= 80	Within Run		Total	
Mean (µmol/L)	SD	CV%	SD	CV%
42.4	1.772	3.7	2.365	5.0
192	1.867	0.9	5.569	2.8

Method Comparison[7]

A comparison of this Beckman Coulter ammonia method (Method 1) vs and a substantially equivalent predicate assay (Method 2) was run per CLSI EP09-A2 using 79 patient samples. The resulting data is as follows:

Correlation Coefficient:	r = 0.999
Regression equation:	Method 1 = 1.00x - 2.5
Range of patients:	27 - 608 µmol/L

Lower Limit of Detection[8]

The lower limit of detection was determined using the formula where:

LOD = LOB + 2SDWR
LOB = Limit of Blank
SDWR = Within Run standard deviation of a low level sample
When run as recommended the lowest limit of detection is 7.1 µmol/L.

REFERENCES

1. Clinical Chemistry Infobase: A Scientific & Management Cyclopedia. Pesce- Kaplan Publishers 1996; 2246-2320.
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. Pesce A.J., Kaplan L.A., eds., Methods in Clinical Chemistry, Mosby, 1987, p, 1091.
6. Evaluation of Precision Performance of Quantitative Measurement Methods, CLSI EP5-A2, 2004.
7. Method Comparison and Bias Estimation Using Patient Samples, CLSI EP09-A2, 2002.
8. Protocols for Determination of Limits of Detection and Limits of Quantitation, CLSI EP17-A2, 2012.

SYMBOLS

Rx ONLY	Prescription Use Only
IVD	*In Vitro* Diagnostic Medical Device
EC REP	Authorized Representative in the European Community
LOT	Batch Code / Lot Number
REF	Catalogue Number
	Consult Instructions for Use
REAG	Reagent
STD	Standard
	Temperature Limitation
	Use by / Expiration Date
	Manufacturer

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