EN

REF 17660

Ammonia Ultra

Enzymatic determination of ammonia in plasma

REAGENT 1: 3 x 20 mL - **STANDARD**: 1 x 5 mL

IVD

CE

STANDARD/CALIBRATOR: the term refers to the standard / the calibrator

REAGENT: the term refers to the single reagent **CONTROL**: the term refers to the control

SUMMARY

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. Studies have shown that excess ammonia can have a toxic effect on the central nervous system and clinical manifestations are typically neurological disturbances. Elevated ammonia may also be observed in severe liver failure as may occur in Reye's Syndrome, viral hepatitis or cirrhosis.

PRINCIPLE

Ammonia, in the presence of glutamate dehydrogenase (GLDH), combines with α-ketoglutarate and NADH to yield glutamate and NAD^+. The decrease in absorbance (NADH ---> NAD^+) at 340 nm is proportional to the ammonia concentration in the examined plasma. The reagent contains lactate dehydrogenase (LDH) in excess, to rapidly reduce endogenous pyruvate so that it does not interfere with the assay system.

REAGENTS

Reagents, stored at 2-8 °C in unopened vials, are stable up to the expiry date indicated on the package.
Reagents must be limpid; do not use if turbid.
Components of the kit and initial concentration of reactive components:

- **REAGENT 1**
  tris buffer 100 mmol/L pH 8.7, α-ketoglutarate 7.5 mmol/L, NADH > 0.2 mmol/L, GLDH > 4000 U/L, LDH > 30000 U/L
- **STANDARD**
  Ammonia Standard 500 µg/dL (294 µmol/L)

Barcode and bottle code number, if printed on reagent labels, are referred to the use of the product on Hitachi 911/912 analyzers. Please refer to the application and detailed information available upon request.

NOTES AND LIMITATIONS

REAGENTS PECULIAR INFORMATION:

- The method traceability is verified using an internal standard obtained by purified material.
- REAGENT 1 must be limpid; do not use if turbid.
- Sources of contamination include (but are not restricted to) cigarette smoking (patient and collection staff), laboratory atmosphere and laboratory glassware.

PREPARATION OF REAGENT SOLUTIONS

REAGENT 1: ready to use. Reagent in unopened vial is stable up to expiry date indicated on the package. Stability: 15 days at 2-8 °C after opening, if contamination avoided.
STANDARD: ready to use. Reagent in unopened vial is stable up to expiry date indicated on the package.

Stability: 120 days at 2-8 °C after opening, if contamination avoided.
It is recommended to re-cap the REAGENT 1 and STANDARD vials if not in use.

QUALITY CONTROL

The use of following control materials at different levels of analyte is recommended to verify test accuracy:

Ammonia Controls **REF 16635** **3x(1x5)mL**
Liquid controls at 3 different levels of analyte in proteic matrix. For use, follow the instructions contained in the kit.

SAMPLE

Plasma (heparin or EDTA). Do not use ammonium heparin. Haemolysed samples should not be used as erythrocytes contain level of ammonia approximately 3 times that of plasma. 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.
Collect samples in accordance with the NCCLS procedure reported in bibliography.[1] Stability of the sample: 3 hours at 2-8 °C or 24 hours at -20 °C.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Safety Data Sheets are available at www.sentineldiagnostics.com or contact your local representative.
- ⚠ **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens,[2] Biosafety Level 2[3] or other appropriate biosafety practices[4,5] should be used for materials that contain or are suspected of containing infectious agents.

INSTRUMENTATION AND MATERIALS REQUIRED BUT NOT PROVIDED

- Usual laboratory equipment
- Filters photometer or spectrophotometer

ANALYTICAL PROCEDURE

Wavelength:	Main = 340 nm / Reference= 600 nm
Pathlength:	1 cm
Temperature:	37 °C
Sample/REAGENT 1:	1/11
Reaction:	fixed time (decrease)

Allow reagents to reach working temperature before using. A proportional variation of the reaction volumes indicated in the analytical procedure does not change the result.

EXAMPLE OF ANALYTICAL PROCEDURE ON AUTOMATED INTRUMENTS

Calibrator / Controls / Sample = 30 µL

R1 = 330 µL	I° reading	II° reading
Time 0	after 60 seconds read the absorbance of the sample (A1S) and the absorbance of the standard (A1ST)	after 120 seconds read the absorbance of the sample (A2S) and the absorbance of the standard (A2ST)

CALCULATION

[(A2S - A1S) / (A2ST - A1ST)] x 500 = µg of ammonia /dL of sample

CONVERSION FACTOR

Ammonia: [µg/dL] x 0.588 = ammonia [µmol/L]

REFERENCE VALUES
Plasma: 31 - 123 µg/dL (18 - 72 µmol/L)

It is recommended that each laboratory establish its own expected range.

PERFORMANCES (determined on automatic analyzer)

Interferences: the test is not affected by the presence of bilirubin up to 20 mg/dL, ascorbic acid up to 40 mg/dL, triglycerides up to 700 mg/dL, piruvate up to 0.75 mmol/L and ALT up to 4000 U/L. Haemoglobin: haemolysed samples should not be used as erythrocytes contain level of ammonia approximately 3 times that of plasma.

Measuring range: 25 - 1700 µg/dL. Samples with concentration higher than 1700 µg/dL must be diluted 1:10 with distilled water and result multiplied by 10.

Intra-Assay Precision: it was determined on 20 replicates of each control (3 levels - L1/L2/L3). Results were as follows:
L1: average 62.30 µg/dL, SD 3.08, CV% 4.94 / L2: average 262.90 µg/dL, SD 10.61, CV% 4.04 / L3: average 366.75 µg/dL, SD 7.46, CV% 2.03.

Inter-Assay Precision: it was determined for 10 days on 2 replicates of each control - 3 different levels (L1/L2/L3). Results were as follows:

	Mean µg/dL	DS Within Run	CV%	SD Run to Run	CV%	SD Tot.	CV%
L1	57.20	3.79	6.63	2.90	5.07	4.78	8.35
L2	269.60	3.00	1.11	2.85	1.06	4.14	1.53
L3	368.63	7.29	1.98	1.79	0.49	7.50	2.04

Sensitivity: 25 µg/dL. Sensitivity was calculated on 10 replicates of normal saline and reported as the "mean zero value + 3 SD".

Accuracy: this test (y) was compared with a commercially available method (x). Results were as follows:
N = 65, r = 0.999, y = 0.993 x + 2.53

WASTE MANAGEMENT
Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

1) NCCLS Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard - Fifth Edition (H3-A5). Wayne, PA: The National Committee for Clinical Laboratory Standards, 2003.
2) US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. Bloodborne Pathogens.
3) US Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories, 5th Ed. Washington,DC: US Government Printing Office, January 2007.
4) World Health Organization. Laboratory Biosafety Manual, 3rd ed.Geneva: World Health Organization, 2004.
5) Sewell DL, Bove KE, Callihan DR, et al. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Third Edition (M29-A3). Wayne, PA: Clinical and Laboratory Standards Institute, 2005.
6) Kaplan, L.A., Pesce, A.J.: “Clinical Chemistry”, Mosby Ed. (1996).
7) EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of Good Laboratory Practice as specified in Council Directive 87/18/EEC.
8) Tietz NW, editor. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders; 1995.
9) "The Diagnosis of Urea Cycle Disorder", Lab Medica International, 13-17, (May-June - 1993).

Explanation of symbols

REAGENT	The term refers to the single reagent
IVD	*In vitro* Diagnostic Medical Device
REF	Catalogue number
LOT	Batch code
Cont.	Contents of kit
	Caution, consult accompanying documents
	Consult instructions for use
	Use by (last day of the month)
xxx	Contains sufficient for <n> tests
	Temperature limitation
	Manufacturer

Note: changes in comparison to the previous version are indicated by a vertical bar in the text margin.