BioMed- AMMONIA II

Enzymatic UV (Monoliquid)

REF:

INTENDED FOR USE:

For the quantitative determination of Ammonia (NH_3) in plasma.

PRINCIPLE:

The enzymatic assay where ammonia combines with α-ketoglutarate and NADPH in the presence of Glutamate Dehydrogenase (GLDH) to produce glutamate and $NADP^+$ is the basis of ammonia. These results in a decrease in absorbance measured at 340 nm which is proportional to the concentration of ammonia in plasma.

$$\alpha\text{-ketoglutarate} + NH_3 + NADPH \xrightarrow{GLDH} \text{glutamate} + NADP^+$$

Clinical Significance:

Increased levels of ammonia (Hyperammonemia) is evident in several inherited and acquired diseases. The inherited deficiencies of urea cycle enzymes is the major cause in infants. The acquired causes are advanced liver disease and renal failure.

Reagent Concentration:

Ammonia Reagent	Tris Buffer	100 mmol/l
	NADPH	0.35 mmol/l
	Sodium Azide	0.1%
	GLDH	1000 KU/L
	α-ketoglutarate	8.0 mmol/l
Calibrator	Ammonium Sulfate	Lot Specific

Reagent Handling and Preparation:

Reagents and calibrators are ready to use.
Unopened bottles are stable up to the expiry date at 2-8°C. Once opened the reagent is stable for a period of 5 days when stored at 2-8°C.

Calibration Frequency:

Two point calibration is recommended:
• Every 24 hours
• After reagent bottle change
• After reagent lot change
• As required following quality control procedures
Calibration verification: Not necessary.

Specimen:

EDTA plasma, non-haemolysed. Draw the specimen from a stasis-free vein and centrifuge in a stoppered tube as soon as possible.
Stability: Place specimen on ice and assay immediately.
Separated plasma: 3 hours at +4°C in a stoppered container
Centrifuge samples containing precipitate before performing the assay.

Stability:	3 hours at +4°C

Manual Procedure:

Wavelength:	340nm
Cuvette:	1cm light path
Temperature:	+37°C
Measurement:	Against air or distilled water

Pipette into test tubes as follows:		
	Reagent Blank	**Sample / Control / Calibrator**
Sample / Calibrator (ST)	---	100 ul
Ammonia Reagent (R)	1000 ul	1000 ul
Mix and immediately measure A1, start the stop watch and measure A2 after exactly 2.5 minutes.		

Calculation:

$\Delta A = A_1 - A_2$

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}} \times \text{Calibrator conc.} = \text{Ammonia conc. (µg/dl)}$$

Measuring Range:

Plasma: 9-1700 µg/dl
Specimen dilution
Manually dilute specimens above the reportable range with freshly distilled or deionized ammonia-free water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).
NOTE: Do not report results generated from automatic rerun unless a fresh sample is poured.

Sensitivity:

Detection limit: 9 µg/dl
The lower detection limit represents the lowest measurable substance concentration that can be distinguished from zero. It is calculated as three standard deviations of 21 replicates of the lowest standard.

Imprecision:

Reproducibility was determined using human samples and controls in an internal protocol run n = 21. The following results were obtained:

Intra Assay – Within run			
Sample	**Mean µg/dl**	SD	**CV %**
Sample 1	73	1.22	1.67
Sample 2	152	2.65	1.74

Intra Assay – Between run			
Sample	**Mean µg/dl**	SD	**CV %**
Sample 1	75	1.25	1.66
Sample 2	150	2.42	1.61

Method Comparison:

A comparison of the Liquid Stable Ammonia (y) with a commercial obtainable assay (x) gave the following result:
y = 0.948 x +3.92; r = 0.999

Interference – Limitations:

Criterion: Recovery within ±10% of initial value.
Icterus: No significant interference up to an I index of 60
(approximate conjugated and unconjugated bilirubin: 60 mg/dl)
Haemolysis: No significant interference up to an H index of 50 (approximate haemoglobin concentration: 50 mg/d).
Lipaemia (Intralipid): No significant interference up to an L index of 250 (approximate triglycerides concentration: 500 mg/dl) There is poor correlation between turbidity and triglycerides concentration.

Normal Values:

Male 25-94 µg/dl (14.7- 55.3 µmol/I)
Female 19-82 µg/dl (11.2-48.2 µmol/I)
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, ammonia results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings

Use on Automated Analysers:
This reagent is suitable for use on a range of automated analysers. Specific instructions for these applications are available on request from our technical department.
For automated analysis use

Quality Control:

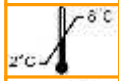
The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Health & Safety:

This kit is designed for use by suitably qualified laboratory personnel only. Exercise the normal precautions required for the handling of laboratory reagents. Do not ingest the material. Dispose of material according to local guidelines.

References:

1. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988; 26:783-790.
2. Berthelot MPE. Repert Chem Appl. 1859:282.
3. Da Fonseca-Wollheim F. Z Klin Chem Klin Biochem. 1973;1 1 :421 ,426.
4. Dokumentation Roche Diagnostics.
5. Hohorst HJ. Biochem Z. 1956;328:507.
6. Kaplan LA, Pesce AJ. Clinical Chemistry Theory, Analysis and Correlation. St. Louis, Mo: CV Mosby Co; 1984:1231.
7. Kirsten E, et al. Biochem Z. 1963;337:312.
8. Passing H, Bablok W. A New Biometrical Procedure for Testing the Equality of Measurements from Two Different Analytical Methods. J Clin Chem Clin Biochem 1983; 21:709-720.
9. Prellwitz W, et al. Med Welt. 1976;27:1277.
10. Siegel JM, Montgomery GA. Arch Biochem/Biophys. 1956;82:288.
11. Tietz NW. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, PA: W.B. Saunders Co; 1995:44.

	Consult Instructions for Use
	Caution, Consult accompanying Documents
IVD	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue Number
LOT	Batch Code
	Use by

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