Ammonia (UV Kinetic Method)

In-vitro Diagnostic reagent kit for quantitative determination of Ammonia activity in plasma samples on Photometric System.

Reagent

Reagent 1: Enzyme Solution
Reagent 2: Substrate Solution
Standard: Ammonia Standard (100 µmol/L)

Principle

The enzymatic determination of ammonia allows a direct measurement of the compound in the plasma which avoids the long and laborious methods of separation employed in older methodologies. Ammonia reacts with α-Ketoglutarate (α-KG) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) to form L-glutamate and NADP in a reaction catalyzed by glutamate dehydrogenase (GLDH) {L-glutamate: NAD(P) + oxidoreductase (deaminating)}. The amount of NADPH oxidized is, on a molar basis, equal to the content of ammonia in the sample. The reaction can be followed by the decrease in absorbance at 340nm. The reagent is provided in two separate vials. Keeping the components of the reagent separated until time of assay increases their stability after reconstitution. The use of NADPH in place of NADH minimizes interference by such components of plasma as pyruvate and lactate dehydrogenase. The enzymatic assay gives a highly sensitive and specific method.

$$NH_4^+ + \alpha\text{-Ketoglutarate} + NAD \xrightarrow{GLDH} \text{L-glutamate} + NAD^+$$

Summary

The bulk of ammonia in the body is generated in the gastrointestinal system by action of bacterial enzymes on the contents of the colon and from hydrolysis of glutamine. It is removed in the liver and converted to urea through a series of enzymatic reactions in the Krevs-Henseleit cycle. Among other conditions, advanced liver disease and hepatic encephalopathy result in elevated levels of ammonia in blood. Hyperammonemia is also common in inherited deficiencies of the enzymes involved in the conversion of ammonia to urea. The determination of ammonia is very useful in the diagnosis and prognosis of Reye's Syndrome. Elevated blood ammonia exerts toxic effects on the central nervous system.

Storage Instructions and Reagent Stability

Reagents and standard are stable up to the end of the indicated month of expiry, if stored at 2°–8°C, protected from light and contamination is avoided. Do not freeze the reagents! Keep the cap tightly closed.

Components and Concentration

Buffer 100 mmol/L, α-Ketoglutarate, NADPH, GLDH, Stabilizer and Preservative.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Mix, 4 parts of Reagent 1 and 1 part of Reagent 2 = Working Reagent.
Protect the reaction solution from light.

Specimen

Heparin Plasma or EDTA plasma separated within 30 minutes after blood collection from cellular contents.
Plasma may be stored for:
2 hours at 2° –8°C

Assay Procedure

Wavelength	340 nm
Optical Path	10 mm
Temperature	37°C

Working reagent	1000 µL
Sample/Standard	100 µL
Mix, incubate for 10sec. and read absorbance (A1). Incubate again for 120 seconds and read absorbance (A2). Calculate the change in absorbance ΔA.	

Calculation

ΔA= (A1-A2) Sample/Standard

ΔA/min and multiply by the corresponding factor from table below:

$$\text{Ammonia [µg/dL]} = \frac{\Delta A \text{ (Sample)}}{\Delta A \text{ (Standard)}} \times 100$$

Quality Controls

For internal quality control any normal and abnormal controls should be assayed with each batch of samples.
Each laboratory should establish corrective action in case of deviations in control recovery.

Warnings and Precautions

1. **Keep out of reach of children. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.**
2. **Take off immediately all contaminated clothing.**
3. **Do not swallow. Avoid contact with skin and mucous membranes.**
4. **For professional use only!**

Performance Characteristics

Measuring Range

The test has been developed to determine the concentration of Ammonia within a measuring range from 5-1500 µmol/L. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Linearity/Limit of Maximum Detection

The higher limit of detection is 1500 µmol/L.

Sensitivity/Limit of Detection

The lower limit of detection is 5 µmol/L.

Interferences

The major interference for this assay is from contamination by ammonia in the air and water. Analytical and physiological variables including drugs and other substances which influence ammonia concentrations have been listed by Young[6,7,8].

Precision

Intra-assay n = 20	Sample 1	Sample 2	Sample 3
Mean[U/L]	20.06	83.76	105.21
SD[U/L]	0.39	0.86	1.21
CV [%]	1.94	1.02	1.15

Inter-assay n = 20	Sample 1	Sample 2	Sample 3
Mean[U/L]	19.26	85.16	104.61
SD[U/L]	0.24	0.69	0.98
CV [%]	1.24	0.81	0.93

Method Comparison

A comparison of Precision Biomed Ammonia (y) with a commercially available test (x) using 15 samples gave following results:
$y = 0.998x + 0.279$; $r^2 = 0.991$

Reference Range

Normal Range (Plasma): 17 - 90 µmol/L
Each laboratory should check if the references range are transferable to its own patient population and determine own reference ranges if necessary.

Quick Reference

Parameter	Ammonia
Mode	Fixed Time Kinetic
Reaction slope	Decreasing
Wavelength	340 nm
Path length	10 mm
Temperature	37° C
Working reagent	4 part Reagent 1
	1 Part Reagent 2
Working Reagent volume	1000 µL
Sample volume	100 µL
Delay	10 Sec.
Reading	120 Sec.
Linearity	1500 µmol/L
Sensitivity	5 µmol/L
Reference range serum	17 to 90 µmol/L

Pack Size

Cat No.	Configuration	Pack
AMM00020	Reagent R1 - 2 x 8mL Reagent R2 - 2 x 2mL Standard – 1 x 3mL	20mL

Literature

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5. Pesh-Imam, M.,Kumar, S.,Wills, C.E., Clin.Chem.,1978;24:2044.
6. Young, D.S., Effects of Pre-analytical Variables on Clinical Laboratory Tests, First Edition, AACC Press, Washington, D.C., 3.20-3.21, 1993.
7. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, Third Edition, AACC Press, Washington, D.C., 3.30-3.32, 1990.
8. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 1991 Supplement to the Third Edition, AACC Press, Washington, D.C., 3.9-3.10, 1991.

Version : AMM /00

IVD In Vitro Diagnostic Use | See Pack Insert For Procedure | Single Use only | CE
Temperature Limit | Manufacturer's Address | Manufacturing Date | Expiry Date | LOT Lot Number

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