AMMONIA

(UV Kinetic Method)

Intended Use

Ammonia is a reagent kit used for the determination of Ammonia in serum or plasma. The reagents are for *in-vitro* diagnostic use.

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. The enzymatic assay gives a highly sensitive and specific method. The assay is based on the following reaction:

$$NH_4^+ + \alpha\text{-KG} + NADPH \xrightarrow{GLDH} L\text{-Glutamate} + NADPH + H_2O$$

Ammonia reacts with α-Ketoglutarate (α-KG) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) to form L-glutamate and NADP ina reaction catalyzed by Glutamate Dehydrogenase (GLDH). 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 340 nm.

Reagent provided

1. R1 – Ammonia Reagent
2. R2 – Ammonia Reagent
3. R3 – Ammonia Standard

Reagent storage and stability

Reagent and standard are stable till the expiry date stated on the container label.

Specimen collection and preservation

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

Assay guidelines for Analyzers

Reaction type	Fixed Time Kinetic
Reaction slope	Decreasing
Wavelength	340 nm
Temperature	37°C
Blank with	Distilled water
Delay Time	30 sec.
Reading Time	180 sec.
Sample volume	100 µl (0.1 ml)
Reagent volume	1000 µl (1.0 ml)
Std. concentration	Refer Std Vial
Low Normal	17 µg/dl
High Normal	120 µg/dl
Linearity	1000 µg/dl

Assay guidelines for Manual procedure

Reagents	Standard (S)	Test (T)
R1 Ammonia Reagent	800 µl (0.8ml)	800 µl (0.8ml)
R2 Ammonia Reagent	200 µl (0.2 ml)	200 µl (0.2 ml)
Bring up the temperature of determination, then add		
Ammonia Standard	100 µl (0.1 ml)	-
Sample	-	100 µl (0.1 ml)

Mix well and after exactly 30 sec read absorbance A1 of the test (T) and standard (S) against water. After next 180 sec repeat absorbance reading (A2) and calculate ΔA (A1-A2) for the test and standard.

Calculation

$$\text{Ammonia Conc.} = \frac{\Delta A\ (T)}{\Delta A\ (S)} \times \text{Conc. of Std.}$$

Normal Range

Guidance value : 17 – 120 µg/dl

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Performance Characteristics

Measuring Range

The test has been developed to determine the concentration of Ammonia up to 1000 µg/dl. When values exceed this value samples should be diluted 1+1 Nacl solution (9g/L) and the resultMultiplied by 2.

Quality Control

To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

References

1. Presh-Imam, M.Kumar, S.Wills, C.E, Clin.Chem.,1978;24:2044
2. Textbook of Clinical Chemistry Edited by N.W. Tietz, W.B. Saunders Company, Philadelphia, p-1409, 1986.
3. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, First Edition, AACC Press, Washington, D.C., 3.20-3.21, 1993.

Symbols

IVD	In Vitro Diagnostics.		Caution.		Keep away from sun light.		Date of Manufacture.
LOT	Batch No.		Read Instructions.		Fragile.		Product Expiry Date.
CONT	Content.		Storage Temperature.		Keep Dry.		Manufactured By.
REF	Catalogue No.						

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