AMMONIA KIT
Mod.GLDH Method

INTRODUCTION

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 commonin 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

METHOD 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

$$NH4+ \alpha\text{-Ketoglutarate} + NADH \xrightarrow{GLDH} Glutamate + NAD + H_2O$$

Ammonia reacts with α-Ketoglutarate (α-KG) and reduced nicotinamide adenine dinucleotide (NADH) to form L-glutamate and NAD in a reaction catalyzed by glutamate dehydrogenase (GLDH) . The amount of NAD oxidized is, on a molar basis, equal to the amount of plasma ammonia in the sample. The reaction can be followed by the decrease in absorbance at 340nm. Interference of endogenous pyruvate is avoided by LDH.

KIT CONTENTS

Reagent name	Pack Size	Pack Size
R1 - Ammonia Reagent	5 x 5 ml	25 x 1 ml
R2 - Ammonia standard	2 ml	2 ml

R2-STANDARD is ammonia standard solution: Please refer the standard value mentioned on the vial.

WORKING REAGENT PREPARATION AND STABILITY

The reagent is ready to use.
The reagent is stable up to the kit expiry date printed on the package when stored at 2-8°C.

CONCENTRATIONS IN THE TEST

Buffer TRIS pH 8.0	81 mmol/l
Oxoglutarate	3.3 mmol/l
NADH	0.18 mmol/l
GLDH (Glutamate dehydrogenase)	4 K U/L
EDTA	4 mmol/l
LDH	2 KU/L

Warnings and notes

- Product for in vitro diagnostic use only.
- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves,glasses).
- In case of contact with skin or yes

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 340 nm;
- Thermostat at 25°C or 37°C;
- General laboratory equipment;

SPECIMEN

EDTA plasma is the specimen of choice. The use of heparin as an anticoagulant is not recommended. Collect blood from a stasis-free vein into an EDTA evacuated tube; release residual vacuum in the tube; mix gently, place on ice anddeliver to the laboratory without delay. Separate the plasma from the cells immediately. Do not use hemolyzed samples. The analysis should be performed within 30 minutes. A maximum of 2 hours delay with the plasma on ice is permissible.

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Programme sheets are available on request.

Wavelength	340 nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Standard (S)	Test (T)
R1 Ammonia Reagent	1000 µl	1000 µl
Bring up the temperature of determination. Then add,		
R2 - Ammonia standard	100 µl	-
Sample		100 µl

Mix well and after exactly 60 sec read absorbance A1 of the test (T) and standard (S) against water. After next 60 sec repeat absorbance reading (A2) and calculate ΔA(A2 – A1) for the test and standard.

CALCULATION

Ammonia concentration = ΔA(T) / ΔA(S) x Standard concentration

REFERENCE VALUES

Plasma - 17 - 90 µg/dl

It is recommended for each laboratory to establish its own reference ranges representing its patient population.

QUALITY CONTROL

To Ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS

Sensitivity / Limit of Quantitation: 0.1 g/dl.

Linearity: up to 1500 µg/dl. For higher concentration of ammonia dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Textbook of Clinical Chemistry Edited by N.W. Tietz, W.B. Saunders Company, Philadelphia, p. 1409, 1986.
2. Ratcliff, C.R. and Hall, F.F. in Selected Methods of Clinical Chemistry,volume 9, p. 85. Edited by Willard R. Faulkner and Samuel Meites. American Association for Clinical Chemistry. Washington, D.C., 1982.
3.Clinical chemistry info base: A scientific and management cyclopedia. pesce- kaplan publishers 1996: 2246-2320.
4.Tietz test book of clinical chemistry. Burtis CA and Ashwood BR(Eds).Second edition ,WB saunders company , 1994: 32: 1485-88.
5. Da Fonseca-Wollheim F., J. Clin. Chem. Clin. Biochem. 11, 421,1973.
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.

SYSTEM PARAMETERS

Method	Fixed time (2-point kinetic)
Wavelength.	340 nm
Zero Setting	Distilled water
Temperature Setting	37°C
Incubation Temperature	37°C
Incubation Time	----
Delay time	60 secs
Read time	60 secs
No. of Reading	2
Interval time	----
Sample Volume	0.1 ml (100 µl)
Reagent Volume	1.0 ml (1000 µl)
Standard Concentration	Refer standard vial
Units	µg/dl
Factor	----
Reaction slope	Decreasing
Linearity	1500 µg/dl

Manufactured by:
Vanguard Diagnostics Private Limited
C-123, Phase-I, Okhla Industrial Area
New Delhi-110 020, INDIA.

Tel: 011-4172 7222,
Email:info@vanguarddiagnostics.com
Website : www.vanguarddiagnostics.com

CE