PATHOZYME DIAGNOSTICS

LIQVIPATH AMMONIA

(Enzymatic - GLDH METHOD)
CAT No.: AMN

Reagent kit for quantitative estimation of Ammonia in Plasma.

DIAGNOSTIC SIGNIFICANCE:

Elevated levels of ammonia may be either due to Inborn errors of metabolism are the major cause of elevated ammonia in infants and usually the result of urea cycle enzyme deficiencies. Inherited disorders affecting the metabolism of the dibasic amino acids (lysine and ornithine) and those involving the metabolism of organic acids may also produce elevated levels of circulating ammonia. Elevated ammonia may also be observed in severe liver failure as may occur in Reye's Syndrome, viral hepatitis or cirrhosis.

PRINCIPLE:

Ammonia reacts with α-Ketoglutarate to form glutamate in presence of glutamate dehydrogenase. NADH is oxidized to NAD+ in this reaction, which is measured as decrease in absorbance at 340 nm. The rate of decrease in absorbance at 340 nm is directly proportional to plasma Ammonia concentration.

$$\text{Ammonia} + \alpha\text{-KG} + \text{NADH} \xrightarrow{\text{GLDH}} \text{Glutamate} + \text{NAD}$$

SPECIMEN COLLECTION:

Plasma: Plasma, collected with EDTA or heparin (not ammonium heparin) into an evacuated collection tube is recommended and placed on ice. Centrifuge (cold) the sample as soon as possible and separate plasma and store at 2-4°C until analysis. Ammonia samples are stable for 3 hours at 2-4°C or 24 hours at –20°C.

KIT PRESENTATION:

Pack Size	2 X 5 ml	5 X 5 ml	5 X 10 ml
R1-Ammonia (Enzyme Reagent)	2 X 4 ml	5 X 4 ml	5 X 8 ml
R2-Ammonia (Starter Reagent)	1 X 2 ml	1 X 5 ml	1 X 10 ml
Ammonia Calibrator	1 X 0.5 ml	1 X 0.5 ml	2 X 0.5 ml

WORKING REAGENT PREPARATION:

Mixing 4 volumes of R1-Ammonia (Enzyme) with 1 volume of R2-Ammonia (Starter). **i.e. 4:1 ratio.**
The Working Reagent is stable for 30 days, It should be tightly capped when not in use, protected and stored at 2-8^{0}C.

AMMONIA Calibrator

Calibrator ready To Use & for value refer the vial label.

REAGENT STORAGE & STABILITY:

All reagents included in the kit are stable at 2-8^{0}C until the expiry date stated on the label.

ASSAY PARAMETERS:

Reaction	: Fix Time	Sample Volume	: 100 µl
Wavelength	: 340 nm	R1 + R2 Volume	: 800 µl + 200 µl
Flow Cell Temp.	: 37^{0}C	Calibrator Conc.	: As on vial
Initial Delay	: 60 Sec	Reaction Slope	: Decreasing
Interval Time	: 90 Sec	Zero Setting	: Dist. Water
Read Time	: 90 Sec	Linearity	: 1500 µg/dl
No. of Reading	: 01	Unit	: µg/dl

PROCEDURE:

Pipette into TT	Calibrator	Test
R1-Ammonia (Enzyme Reagent)	800 µl	800 µl
R2-Ammonia (Starter Reagent)	200 µl	200 µl
Calibrator	100 µl	--
Sample (Test)	--	100 µl

Mix & aspirate immediately and read difference in absorbance between 60 seconds (AT_1) and 150 seconds (AT_2) for Calibrator & Test.

CALCULATION:

$$\text{Ammonia (µg/dl)} = \frac{\Delta\text{Abs of Test} \times \text{Calibrator Conc.}}{\Delta\text{Abs of Calibrator}}$$

Where $\Delta Abs = (AT_1) - (AT_2)$

Unit conversion: µg/dl x 0.5882 = µmol/L

NORMAL VALUES:

Plasma Ammonia**:** 17 - 90 µg/dl

LINEARITY:

The method is **linear up to 1500 µg/dl**. For Ammonia concentration higher than linearity limit, should be diluted **with ammonia free water** and re-assayed. Multiply results by the dilution factor.

REFERENCE:

1. Ektachem Multilayer Dry Film Assay for Ammonia Evaluated. J Clin Chem 1985: Vol 31:12:2012-2014.
2. Clinical Chemistry Infobase: A Scientifi c & Management Cyclopedia. Pesce-Kaplan Publishers 1996; 2246-2320.

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Expiry Date | IVD In-Vitro Diagnostics Use | Storage | Mfg. Date | LOT Batch Number | REF Catalogue Number | See Package Insert

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