AMMONIA

Quantitative enzymatic determination of ammonia in plasma

CLINICAL SIGNIFICANCE

Ammonia is one of the by-products of metabolism of the proteins and is immediately converted by the liver into urea to be eliminated later with urination.
The alteration or malfunction of this process causes the ammonium accumulation in body, which some studies have demonstrated to be toxic to the central nervous system with typical neurological disorders.
The increase of ammonia in the blood is observed in severe liver disorders such as Reye's syndrome, in the terminal phase of particularly serious viral hepatitis and liver cirrhosis. Elevated ammonia levels in the blood have been reported even in the presence of heart failure, azotemia, and pulmonary emphysema.

TEST SUMMARY

The ammonium ($NH4^+$) contained in the plasma sample in the presence of glutamate dehydrogenase (GLDH), reacts with α-ketoglutarate and NADH, forming glutamate and NAD^+ according to the reaction:

NH_4^+ + α-ketoglutarate + NADH $\xrightarrow{GLDH}$ L-Glutamate + NAD^+ + H_2O

The decrease in absorbance is proportional to the concentration of ammonium in the sample and is measured spectrophotometrically at 340 nm.

SAMPLES

Plasma (heparin or EDTA).
Do not use Ammonium Sulfate or Ammonium Heparin.
It is recommended to place the blood sample in ice and centrifuge (cold) as soon as possible, to separate the plasma.
Ammonia in plasma is stable for 2 hours at 2-8°C or 24 hours at -20°C.

Plasma samples should be analyzed within 30 minutes of collection, alternatively store at 2-8°C for 2 hours or at -20°C for 24 hours.
Make sure that the sample tubes are closed tightly to avoid evaporation of ammonia.

REAGENTS

Reagent 1: Tris buffer pH 7.4, GLDH, LDH, ADP-Na2, α-ketoglutaric acid, preservatives and stabilizers.

Reagent 2: Caps Buffer pH 10.25, NADH, Sodium Azide, preservatives and stabilizers.

Standard: Ammonia 500 µg/dL (293,5 µmol/L), preservatives and stabilizers

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. Automatic Micropipette. Cuvettes in optical glass or disposable in optical polystyrene. Distilled water.

PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

REAGENTS PREPARATION

Reagents are liquid, ready to use and stored at 2-8°C away from direct light source.
If not contaminated, and sealed immediately after use, the reagents are stable until the expiration date indicated on the package.

PROCEDURE

Bring the reagents to the working temperature before use.

Kind of analysis:	Kinetics decreasing
Reading time:	6 – 10 minutes
Wavelength:	340 nm
Temperature:	37°C
Zero:	Blank Reagent

Reagents	Blank	Standard	Sample
Distilled water	100 µl	--	--
Standard	--	100 µl	--
Sample	--	--	100 µl
Reagent 1	800 µl	800 µl	800 µl
Mix and incubate for 5 minutes at 37°C. Then add:			
Reagent 2	200 µl	200 µl	200 µl
Mix and incubate at 37°C. After 1 minute, read the absorbance (A1) of the sample and standard at 340 nm against Blank Reagent. Read the second absorbance (A2) exactly 4 minutes after the first reading.			

CALCULATION

$$\text{Ammonia (µg/dL)} = \frac{A1_{sample} - A2_{sample}}{A1_{standard} - A2_{standard}} \times 500$$

Ammonia (µmol/L) = µg/dL x 0.587

EXPECTED VALUES

Adults		
Men	27 - 102 µg/dL	15.8 – 59.9 µmol/L
Women	19 - 87 µg/dL	11.2 – 51.1 µmol/L
Children	< 81.5 µg/dL	< 47.8 µmol/L
Newborns	< 228 µg/dL	< 133.8 µmol/L

Every laboratory should establish own reference intervals in accordance with own population.

NOTE

- It's possible modify the volume of reagents proportionally
- As with any diagnostic procedure, if the results are incompatible with clinical presentation, the physician should confirm the data obtained using this test, with other clinical information.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL

it is suggested to perform an internal quality control.

TEST PERFORMANCE

Precision

Intra-assay (n = 20)	Mean (µg/dL)	SD	CV%
Sample 1	31.3	1.1	4.3
Sample 2	151.4	2.3	3.2

Sensitivity/limit of detection
The method is able to discriminate until 12.0 µg/dL.

Linearity
The method is linear up to 1700 µg/dL.
If the values is exceeded, it is suggested to dilute the sample 1:10 with distilled water, to repeat the test multiplying the results by 10.

Interferences
Lipemic sera or the presence of Ac. Ascorbic can cause interference.
Haemolysed samples should not be used since erythrocytes contain level of ammonia approximately 3 times that of plasma.

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC04300	(50 TESTS)	
Reagent 1	2 x 20 ml	(liquid)
Reagent 2	1 x 10 ml	(liquid)
Standard	1 x 5 ml	(liquid)

REFERENCES

1 Pasquinelli F. e al.; Diagnostica e tecniche di Laboratorio , 2001dd
2 Dewan, J.G. Biochem. J. 1938
3 Mondzac , Ehrlich G.E. , Seegmiller J. E. J. Lab. Clin. Med. 1965

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SYMBOLS

IVD	Only for IVD use
LOT	Lot of manufacturing
REF	Code number
	Storage temperature interval
	Expiration date
	Warning, read enclosed documents
	Read the directions
	Biological risk

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