Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12761 R1 1 x 20 ml
For 24 tests R2 1 x 4 ml
R3 1 x 1.2 ml

Ammonia

Fully enzymatic UV method

Liquid Reagents

Test 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:

$$\alpha\text{-ketoglutarate} + NH_4^+ + NADH \xrightarrow{GLDH} \text{L-glutamate} + NAD^+ + H_2O$$

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

Concentrations in the test

Reagent R1		
Buffer pH = 7.8	50	mmol/L
α-ketoglutarate	6.0	mmol/L
ADP	0.5	mmol/L
LDH	≥ 2	KU/L
EDTA	6.6	mmol/L
Stabilizer		
Reagent R2		
NADH	1.77	mmol/L
Reagent R3		
GLDH	≥ 14	KU/L

Standard : The Concentration as indicated on vial.

Stability and preparation of working reagent

Reagent R1: *liquid.*
Reagent R2: *liquid.*
Reagent R3: *liquid, ready to use.*
All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent:
Mix 5 volumes of reagent R1 with 1 volume of reagent R2.
Stability : 2 weeks at 2 - 8 °C.

Note: The reagent solutions should be clear, cloudiness indicates contamination and reagents should be discarded

Specimen collection and handling

1. EDTA plasma is the specimen of choice.
2. Collect blood from a stasis-free vein into an EDTA evacuated tube, release residual vacuum in the tube, mix gently, place on ice till time of analysis.
3. Separate the plasma from the cells immediately. Don't use hemolyzed samples.
4. Plasma may be stored tightly sealed for up to 2 hours, provided the sample is kept on ice or refrigerated.
5. Serum is not an acceptable specimen.

Standard

Ammonia STD. Cat. No. 16031

Quality control

Ammonia/Ethanol control level 1 Cat. No. 15191
Ammonia/Ethanol control level 2 Cat. No. 15201

Procedure

Wavelength	Hg 340 nm (334 - 365 nm)
Spectrophotometer	340 nm
Cuvette	1 cm light path
Temperature	20 - 25 °C
Measurement	against distilled water.
Reaction	Fixed time

Assay

	Sample (plasma)	Standard
Sample (plasma)	200 µl	--
Standard	--	200 µl
Working Reagent	1000 µl	1000 µl
Mix, incubate for 4 min. Read the absorbance (A_1) against distilled water. The reading (A_1) must be corrected to compensate for the volume addition of reagent R3. **(A_1 x 0.96 = A1c)**		
Reagent R3	50 µl	50 µl
Mix, incubate for 5 min. Read the absorbance (A_2) against distilled water. Calculate the change in absorbance ΔA. **ΔA= A1c – A_2**		

Calculation:

Assay values can be obtained with the test procedure by using a factor or by using ammonia standard.

Factor:

$Conc._{Ammonia}$ (µmol/L) = ΔA_{Sample} X Factor

Factor = 1005 (at 340 nm)

Standard:

$$Conc._{Ammonia}\ (\mu mol/L) = \frac{\Delta A_{Sample}}{\Delta A_{Standard}} \times Conc._{Standard}\ (\mu mol/L)$$

µmol/L ⟷ (X 1.703 / 0.587 X) µg/dl

Linearity

Up to 600 µmol/L (10.2 mg/L).
If the result exceeds 600 µmol/L, repeat the test using diluted serum (1+2) with freshly distilled or deionised ammonia-free water and multiply the result by 3.

Medichem Middle East
Aleppo, Syria, E-mail: info@medichem-me.com

Reference range

1 d.		< 245 µg/dl	< 143.8 µmol/L
5 – 6 d.		< 228 µg/dl	< 133.8 µmol/L
Children		< 82 µg/dl	< 48.10 µmol/L
Adults	women	< 82 µg/dl	< 48.10 µmol/L
	men	< 94 µg/dl	< 55.17 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.

Precautions

1. Avoid ammonia contamination from air, water, and glassware.
2. Through-traffic and smoking must be avoided in the patients room and in the laboratory where the assay is performed. The phlebotomist should be a nonsmoker. If the patient is a smoker, wash site of venipuncture. Blood should be drawn in a room where no smoking is permitted.
3. WARNING: This product contains < 0.1 % sodium azide. Sodium azide may react with lead or copper plumbing to form explosive compounds. When disposing this product through plumbing fixtures, flush with large amounts of water to prevent azide build up.
4. This reagent should be kept closed to avoid ammonia contamination from the laboratory environment. Heavy metals will also interfere in the reaction by inhibiting GLDH.

References:

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Medichem Middle East Aleppo, Syria, E-mail: info@medichem-me.com