BIOLABO
www.biolabo.fr
MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

AMMONIA Enzymatic method

Reagent for quantitative determination of ammonia in human plasma.

REF 99261:	**R1** 6 x 20 mL	Coenzyme-Buffer
	R2 1 x 1.5 mL	Enzymes
	R3 1 x 10 mL	Standard 500 µg/dL

TECHNICAL SUPPORT AND ORDERS
Tel : (33) 03 23 25 15 50
Fax: (33) 03 23 256 256

CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1)

There are several causes, either inherited or acquired, of hyperammonemia. The inherited deficiencies of urea cycle enzymes are the major cause of hyperammonemia in infants. The acquired causes of hyperammonemia are advanced liver disease and renal failure (acute hepatitis, cirrhosis and Reye's syndrome).

PRINCIPLE (4) (5)

Enzymatic method for determining ammonia concentration in plasma, described by MONZAC and Al, modified by VAN ANKEN and Al.

The reaction scheme is as follows:

$$NH_4^+ + \text{Oxoglutarate} + NADPH + H^+ \xrightleftharpoons{GLDH} \text{Glutamate} + NADP^+ + H_2O$$

The decrease in absorbance due to the oxidation of NADPH into $NADP^+$ is proportional to the amount of ammonia in plasma and is measured at 340 nm.

REAGENTS COMPOSITION

Vial R1 **COENZYME-BUFFER**

Buffer TRIS pH 8.0 at 25°C	81	mmol/L
Oxoglutarate	3.3	mmol/L
NADPH	0.18	mmol/L
EDTA	≥ 4	mmol/L
Preservative		

Before reconstitution:
T , R23/24/25: Toxic by inhalation, in contact with skin and if swallowed
S22 - 28$_3$: Do not breathe dust. After contact with skin, wash immediately with plenty of water and soap.
S37 - S38: Wear suitable gloves. In case of insufficient ventilation, wear suitable respiratory equipment.

Once reconstituted: None

Vial R2 **ENZYME**

GLDH (Glutamate dehydrogenase)	≥ 9300	IU/L

Vial R3 **STANDARD NH_3** 500 µg/dL (294 µmol/L)

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENT PREPARATION

Vials R2, R3: Reagents are ready for use.

Vial R1: Add promptly 20 mL of demineralised water.

Mix gently and wait for complete dissolution before using reagents (approximately 2 minutes).

STABILITY AND STORAGE

Store away from light, well cap in the original vial at 2-8°C.

- Standard (vial R3): Transfer the requested quantity, recap and store at 2-8°C.
- Unopened, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Once reconstituted, working reagent (vial R1) is stable for 1 month when free from contamination.
- Once opened, reagent R2 is stable for 6 months when free from contamination.
- Discard any reagent if cloudy or if reagent blank at 340 nm is < 0.600
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2)

Plasma (EDTA or heparin other than ammonium heparinate).

Collect blood from a stasis-free vein into an evacuated tube avoiding partial filling.

Put on ice and centrifuge within 15 minutes in a stopper tube. Separate plasma and perform the analysis immediately to avoid overestimated ammonia results.

INTERFERENCES (3) (5)

Studies on sera show results as follows::

Interferent	Ammonia in specimen (µg/dL)	Results
Ascorbic acid	200	No interference up to 25 mg/dL
Total Bilirubin	158	Positive interference above 10 µmol/L
Haemoglobin	193	Negative Interference above 37 µmol/L
Glucose	121	No interference up to 1000 mg/dL
Lipemia	176	No interference up to 0.304 abs measured at 600 nm (lactescence)

Do not use hemolysed specimens.
To avoid contamination by environmental ammonia, promptly close vial of reagents and Standard after use.
The interference of heavy metals is limited by EDTA in reagent.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control

CALIBRATION

- Standard enclosed in the kit (vial R3) measured on standardized conditions with enzymatic method and aqueous standard traceable to NERL Standard
- Or any calibrator traceable on a reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of reagent.

It is recommended to calibrate in the following cases:
1. When using a new batch of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh serum.

QUALITY CONTROL

- Normal Control Ethanol Ammonia Bicarbonate REF 95013
- Pathological Control Ethanol Ammonia Bicarbonate REF 95023
- External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

NH_3	µg/dL	[µmol/L]
0-10 days	170-341	[100-200]
10 days to 2 years	68-136	[40-80]
≥ 2 years	19-60	[11-35]

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES CHARACTERISTICS

Within-run N = 20	*Level I*	*Level II*
Mean µg/dL	86.2	500
S.D. µg/dL	3.2	3.9
C.V. %	3.7	0.78

Run to run N = 20	*Level I*	*Level II*
Mean µg/dL	201.4	499.2
S.D. µg/dL	7.05	19.6
C.V. %	3.5	3.92

Detection limit: approximately 45.3 µg/dL

Sensitivity for 500 µg/dL of NH_3: approximately 0.115 Abs. at 340 nm

Comparison studies with a commercially available reagent (enzymatic method): No data yet available

LINEARITY

The assay is linear up to 2000 µg/dL (1175 µmol/L).
Above, dilute specimen (1 + 4) with demineralised (ammonia-free) water and reassay taking into account dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Pipette into well identified test tubes:	**Blank**	**Standard**	**Assay**
Plasma			100 µL
Standard R3		100 µL	
Demineralised water	100 µL		
Reagent 1	1,5 mL	1,5 mL	1,5 mL
Mix. Allow to stand for 1 minute at room temperature. Read **absorbance A1** at 340 nm against water.			
Reagent 2	10 µL	10 µL	10 µL
Mix. Allow to stand for 10 minutes at room temperature. Read **absorbance A2** at 340 nm against water.			

Note: Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

$$\text{Ammonia} = \frac{(A1 - A2)_{\text{Assay}} - (A1 - A2)_{\text{Blank}}}{(A1 - A2)_{\text{Standard}} - (A1 - A2)_{\text{Blank}}} \times \text{Standard concentration}$$

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1146-1147.*
(2) *Clinical Guide to Laboratory Test, 4Th Ed., N.W. TIETZ (2006) p. 98-99.*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-40 à 3-42*
(4) *MONDZAC A, EHRLICH GE, and SEEGMILLER JE.: An enzymatic determination of Ammonia in biological fluids. The journal of laboratory and Clinical medicine (J. Lab. Clin. Med.) Vol 66 1965 pp. 526-531.*
(5) *VAN ANKEN H.C. and SCHIPHORST M.E. A kinetic determination of Ammonia in Plasma. Clinica Chimica Acta, 56 (1974) pp. 151-157*

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with