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**Liquid Reagents – ready to use**

# Ammonia

**Enzymatic, UV**

Single Reagent

**Diagnostic Reagent for quantitative in vitro determination of Ammonia in human plasma on photometric systems.**

| REF | Kit Size | Configuration |
|---|---|---|
| **N08160** | **5 x 10 mL** | Single Reagent |
| **N49911** | **1 x 50 mL** | Single Reagent |
| **NA0804** | **5 x 20 mL** | Single Reagent |
| **NT1004** | **5 x 20 mL** | Single Reagent |
| **NE1804** | **5 x 20 mL** | Single Reagent |

Additionally offered:

| | | |
|---|---|---|
| Y08310SV | 1 x 5 mL | Ammonia Standard |
| Y08330 | 2 x 5 mL | Ammonia Control Set (2 levels) |

## TEST PARAMETERS

| | |
|---|---|
| Method: | enzymatic, UV, 2-point kinetic, decreasing reaction |
| Wavelength: | 340 nm, 380 nm |
| Temperature: | 37°C |
| Sample: | EDTA plasma or heparinized plasma (not ammonium heparin!)<br>(serum is not recommended) |
| Linearity: | up to 1174 µmol/L |
| Sensitivity: | The lower limit of detection is 4.1 µmol/L |

## SUMMARY [1-4]

Circulating ammonia in normal individuals is relatively low, despite the fact that ammonia is continuously produced from dietary and amino acid metabolism. Blood ammonia measurments have been used in the diagnosis of coma associated with hepatic dysfunction caused by cirrhosis and neoplasms. The measurement of ammonia is very useful in the diagnosis and prognosis of Reye's Syndrome.
The assay of blood ammonia has always been a tedious and time consuming process. Ammonia assays have generally been based on two approaches: the diffusion of ammonia from an alkaline medium with trapping in acid [1] or separation of ammonia from a sample using ion exchange resin [2].
This assay is an enzymatic method [3] which requires no sample preparation and employs glutamate dehydrogenase and a stabilized NADPH analogue [4] which is easy to use and applicable to routine instrumentation.

## TEST PRINCIPLE

Ammonia reacts with α-ketoglutarate and reduced cofactor to form L-glutamate and the cofactor. The reaction is catalyzed by glutamate dehydrogenase. The decrease in absorbance due to the oxidation of the reduced cofactor can be monitored at 340 or 380 nm and is proportional to the ammonia concentration.

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

## REAGENT COMPOSITION

| COMPONENTS | CONCENTRATION |
|---|---|
| Buffer, pH 8.0 | |
| alpha-ketoglutarate, | 10 mmol/L |
| GLDH (microbial) | ≥ 24 KU/L |
| NADPH analogue | 0.2 mmol/L |
| stabilizers, preservative, detergent | |

## REAGENT PREPARATION

The reagent provided is ready for use.

## REAGENT STABILITY AND STORAGE

| | |
|---|---|
| Conditions: | protect from light<br>close immediately after use |
| Storage: | at 2 – 8 °C |
| Stability: | up to the expiration date |

The reagent should be clear. Turbidity would indicate deterioration.

## SAMPLE STABILITY AND STORAGE

Use fresh, clear, unhemolysed EDTA or heparinized plasma (not ammonium heparin). Serum is not an acceptable specimen.
The accuracy of ammonia determination is extremely dependent on sample collection.
Plasma collected into an EDTA or heparin (not ammonium heparin) evacuated tube is recommended. Release the residual vacuum immediately, place the sample on ice, and deliver to the lab as quickly as possible. Separate the plasma from the sample without delay. Samples should be analyzed within 30 minutes. If this is not possible, samples may be stored tightly sealed:

Stability (tightly sealed): at 2 – 8°C 2 hours [5]
Discard contaminated specimens.
Do not use hemolyzed or turbid samples.

## MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

## MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

| Pipette into test tubes | Blank | Standard | Sample |
|---|---|---|---|
| Reagent | 1000 µl | 1000 µl | 1000 µl |
| Sample | - | - | 100 µl |
| Std./Cal. | - | 100 µl | - |
| dist. $H_2O$ | 100 µl | - | - |
| Mix. Incubate 30 seconds at 37°C and read A1.<br>Incubate for further 10 minutes 37°C and read A2.<br>Calculate:<br>ΔA = [(A2 - A1) sample or std.] – [(A2 - A1) blank] | | | |

## CALCULATION

$$\text{Ammonia (µmol/L)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ std.}} \times \text{conc. of std. (µmol/L)}$$

## UNIT CONVERSION

µg/mL x 58.71 = µmol/L

## REFERENCE RANGE [3] *

| | µmol/L | µg/mL |
|---|---|---|
| Adults: | 12 – 47 | 0.20 – 0.80 |

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

## PERFORMANCE CHARACTERISTICS

### LINEARITY, MEASURING RANGE

The assay is linear from 8.8 to 1174 µmol/L (0.15 – 20.0 µg/mL). A sample with an ammonia level exceeding the linearity limit should be diluted with 0.9% saline and reassayed multiplying the result by the dilution factor.

### SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 4.1 µmol/L (0.07 µg/ml).

### PRECISION (at 25°C)

| Intra-assay n = 20 | Mean [µmol/L] | SD [µmol/L] | CV [%] |
|---|---|---|---|
| Sample 1 | 28.2 | 1.06 | 3.7 |
| Sample 2 | 139.7 | 1.82 | 1.3 |
| Sample 3 | 298.3 | 1.64 | 0.5 |

| Inter-assay n = 20 | Mean [µmol/L] | SD [µmol/L] | CV [%] |
|---|---|---|---|
| Sample 1 | 29.4 | 1.12 | 3.7 |
| Sample 2 | 92.8 | 1.23 | 1.3 |
| Sample 3 | 298.2 | 2.11 | 0.7 |

## SPECIFICITY/INTERFERENCES

no interference up to:

| | |
|---|---|
| Ascorbic acid | 3 mg/dL |
| Bilirubin | 40 mg/dL |
| Triglycerides* | 600 mg/dL |
| Pyruvate | 6.6 mg/dL |
| Lactate | 200 mg/dL |

* At ammonia concentration 121.5 µmol/L
At lower ammonia levels, Triglycerides interfere in even minimal concentrations.

Avoid ammonia contamination from the laboratory environment. Heavy metals will interfere in the reaction by inhibiting GLDH. Hemolysed samples should not be used as erythrocytes contain larger amounts of ammonia than are found in plasma [7].

For further information on interfering substances refer to Young DS [7].

## METHOD COMPARISON

A comparison between Dialab Ammonia (y) and a commercially available assay (x) using 40 samples gave following results: y = 0.997 x + 8.8 µmol/L; r = 1.000

## QUALITY CONTROL

All controls with ammonia values determined by this method can be used.
We recommend the Dialab **Ammonia Control Set** (2 levels).
Each laboratory should establish corrective action in case of deviations in control recovery.

## CALIBRATION

The assay requires the use of an ammonia standard or an ammonia calibrator.
We recommend the Dialab **Ammonia Standard**.

## AUTOMATION

Special applications for automated analysers can be made on request.

## WARNINGS AND PRECAUTIONS

1. Avoid contact with skin and eyes.
2. Please refer to the safety data sheet and take the necessary precautions for the use of laboratory reagents.
3. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
4. For professional use only!

## WASTE MANAGEMENT

Please refer to local legal requirements.

## REFERENCES

1. Conway, E.J., Biochem J. 29:27 (1935).
2. Kingsley, G.R., and Tager H.S., Standard Methods of Clinical Chemistry 6:115, Washington D.C. 1970, American Assoc. of Clinical Chemistry.
3. Ratcliff, C.R., and Hall, F.F. Selected Methods of Clinical Chemistry 9:85, Edited by Willard R. Faulkner and Samual Meites, American Association for Clinical Chemistry, Washington D.C. (1982).
4. U.S. Patent No. 5,801,006.
5. Tietz, N.W., Fundamentals of Clinical Chemistry, 3rd Edition, W.B. Saunders, Philadelphia, PA (1999).
6. CLSI Guidelines and Standards, Clinical and Laboratory Standards Institute, Wayne, PA.
7. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, AACC Press, Washington, Third Edition (1990).

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