AMMONIA UV

INTENDED USE

This reagent is intended for the enzymatic measurement of ammonia in plasma. For *in vitro* diagnostic use only.

CLINICAL SIGNIFICANCE

The bulk of ammonia in the body is generated in the gastrointestinal system by action of bacterial enzymes on the contents of the colon and from hydrolysis of glutamine. It is removed in the liver and converted to urea through a series of enzymatic reactions in the Krevs-Henseleit cycle. Among other conditions, advanced liver disease and hepatic encephalopathy result in elevated levels of ammonia in blood. Hyperammonemia is also common in inherited deficiencies of the enzymes involved in the conversion of ammonia to urea. The determination of ammonia is very useful in the diagnosis and prognosis of Reye's Syndrome. Elevated blood ammonia exerts toxic effects on the central nervous system.

METHOD AND 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 :

$$NH_4^+ + \alpha\text{-KG} + NADPH \xrightarrow{GLDH} \text{L-glutamate} + NADP^+ + H_2O$$

Ammonia reacts with α-Ketoglutarate (α-KG) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) to form L-glutamate and NADP in a reaction catalyzed by glutamate dehydrogenase (GLDH) {L-glutamate: NAD(P) + oxidoreductase (deaminating), EC 1.4.1.3}. The amount of NADPH oxidized is, on a molar basis, equal to the content of ammonia in the sample. The reaction can be followed by the decrease in absorbance at 340nm. The reagent is provided in two separate vials. Keeping the components of the reagent separated until time of assay increases their stability after reconstitution. The use of NADPH in place of NADH minimizes interference by such components of plasma as pyruvate and lactate dehydrogenase.

REAGENT COMPOSITION

Ammonia Substrate/Reagent 1	
Buffer	100 mmol/L
EDTA	2 mmol/L
α-Ketoglutarate	3.4 mmol/L
Adenosine diphosphate	0.5 mmol/L
NADPH	0.3 mmol/L
Fillers and Stabilizers	
pH=8.6 ± 0.1	
Ammonia Enzyme/Reagent 2	
Buffer	100 mmol/L
EDTA	2 mmol/L
Adenosine diphosphate	0.5 mmol/L
GLDH	400 KU/L
Fillers and Stabilizers	
pH=7.8 ± 0.1	
Ammonia Std	**500 microgram/dl**

WARNINGS AND PRECAUTIONS.

1. Avoid ammonia contamination from the air, water, and glassware. Ammonia contamination can be checked by assaying the water used with these reagents. Run a blank assay by substituting the water used for the sample.
2. Through-traffic and smoking must be avoided in the patient's room and in the laboratory where the assay is performed. The phlebotomist should be a non-smoker. If the patient is a smoker, wash site of venipuncture. Blood should be drawn in a room where no smoking is permitted.
3. Do not use reagent if the absorbance of Reagent read at 340 nm against a blank of water is less than 1.000.
4. Avoid contaminating reagents. If reagent shows microbial contamination, as indicated by turbidity, do not use.
5. WARNING: This product contains < 0.1% sodium azide. Sodium azide may react with lead or copper plumbing to form explosive compounds. When disposing of this product through plumbing fixtures, flush with large amounts of water to prevent azide build up.

REAGENT PREPARATION.

The working reagent is prepared by mixing (4) volumes of R1 with (1) volume of R2 in a disposable container.

STORAGE AND STABILITY OF REAGENT.

Store reagent set at 2-8°C (refrigerated). The reagents are stable until the expiration date if stored as directed. Protect from direct light. Avoid microbial contamination.

REAGENT DETERIORATION

The reagent should be discarded if:

1. Turbidity has occurred; turbidity may be a sign of contamination.
2. The working reagent has an absorbance against water less than 1.000 at 340 nm.

SPECIMEN COLLECTION AND STABILITY

EDTA plasma is the specimen of choice. The use of heparin as an anticoagulant is not recommended. Collect blood from a stasis-free vein into an EDTA evacuated tube; release residual vacuum in the tube; mix gently, place on ice and deliver to the laboratory without delay. Separate the plasma from the cells immediately. Do not use hemolyzed samples. The analysis should be performed within 30 minutes. A maximum of 2 hours delay with the plasma on ice is permissible.

INTERFERING SUBSTANCES

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.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.

Wavelength:	340nm
Temperature:	37°C
Cuvettes (square):	1 cm light path
Reagent 1:	0.8 mL
Sample:	0.1 mL

1. Add: 0.2ml Reagent 2. Mix gently and immediately aspirate into the analyzer. After 30 seconds of aspiration read absorbance A1 . After 180 seconds of reading A1 read A2 calculate the , ΔA. Use this ΔA value in the calculation below.

   ΔA = A1 – A2

LIMITATIONS

Samples with ammonia concentrations exceeding 1000 micro g/dL should be assayed again after dilution with an equal volume of distilled or deionized water. Multiply result by 2.

Calibration

Assay values can be obtained with this test procedure by using ammonia standard.

Calculations

$$\frac{\text{Conc std (500 microgram/dl)}}{\Delta A \text{ of Standard}} \times \Delta A \text{ Sample} = \text{micro g/dL ammonia in sample.}$$

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established alkaline phosphatase values may be used for quality control. The assigned value of the control material must be confirmed, by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

The expected range reported for the enzymatic procedure described is upto 120 microgram/dl. It is recommended that each laboratory establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Linearity: 1000 micro gram/dl
2. Comparison: Sixty patient samples were divided into two respective aliquots and stored in an ice bath. The correlation coefficient was 0.999 and the regression equation was: y = 0.991 x – .561.
3. Precision:

Within Run		n=10
Mean	S.D.	C.V.%
94	1.3	1.37
209	1.47	0.70
411	0.92	0.22

General Technical Parameters

Mode	**Fixed time Kinetic**
Wavelength (Filter)	**340 nm**
Reaction Direction	**Decreasing**
Reagent Blank	**NLT 1.000**
Sample Vol.	**100 µl**
Reagent Vol.	**1000 µl**
Delay Time / Lag Time	**30 Sec**
No of readings	**1**
Measuring Time	**180 Sec**
Calibration method	**1-point**
Standard Concentration	**500 microgram/dl**
Linearity	**1000 microgram/dl**
Decimal Places	**0**
Temp.	**37 deg c**
Unit	**microgram/dl**
Ref. Low (Male / Female)	**Upto 10 microgram/dl**
Ref. High (Male / Female)	**Upto 120 microgram/dl**

REFERENCES

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