POINTE SCIENTIFIC, INC.
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Ammonia Reagent Set

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.[1,2]

Test Summary
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[2,3]:

$$NH^{4+} + \alpha\text{-KG} + NADPH \xrightarrow{GLDH} \text{L-glutamate} + NADP^{+} + H_2O$$

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.

Reagents

Ammonia Substrate/Reagent 1	
Buffer	100 mmol/L
EDTA	2 mmol/L
a-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	

Reagent Preparation
Use only ammonia-free water, freshly deionized or distilled. Dissolve Reagent 1 and Reagent 2 with the volume of water specified on the vial label. Maintain solutions tightly capped.

Reagent Storage and Stability
The dry reagents in the unopened vials are stable until the expiration date on the vial labels. The reconstituted reagents are stable for at least 15 days stored at 2°C to 8°C.

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 1 read at 340 nm against a blank of water is less than 1.200.
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.

Specimen Collection and Storage
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.

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.[4, 5, 6]

Materials Provided
Ammonia Reagents in dry powder form in vials to be reconstituted as described above.

Materials Required but not Provided
1. Accurate pipetting devices
2. Matched cuvettes, square, 1 cm light path.
3. Ammonia standards or calibrators.
4. Spectrophotometer able to read at 340 nm.
5. Constant temperature device is not needed. However, use the same temperature for assay of controls and samples.

Ammonia
Reagent Set

Procedure

Wavelength:	340nm
Temperature:	25˚C
Cuvettes (square):	1 cm light path
Blank:	Water
Reagent 1:	1 mL
Sample:	0.2 mL

1. Mix. Incubate for 4 minutes. Read absorbance at 340nm with instrument set to zero absorbance with the water blank. This is reading R1, which must be corrected to compensate for the volume addition of Reagent 2:

   $R_1 \times 0.96 = R_{1c}$

   R_{1c} is used to calculate the ΔA below.

2. Add: 0.05ml Reagent 2. Mix. Incubate. After 5 minutes read absorbance again. This is reading R2. Calculate the change in absorbance, ΔA. Use this ΔA value in the calculation below.

   $\Delta A = R_{1c} - R_2$

Note: Run a blank assay using water in the place of the sample to check for contamination. The value obtained for the blank should then be subtracted from the value found for the sample.

Limitations

Samples with ammonia concentrations exceeding 600 umol/L ($\Delta A > 0.600$) 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 a factor or by using ammonia standards. (Refer to the Calculations section below.) Commercially available ammonia standards or calibrators may be used. Ammonium sulfate standards also can be obtained from the College of American Pathologists or can be prepared from desiccated reagent grade ammonium sulfate.

Calculations

1. Factor

   $$\Delta A \times \frac{1.25 \times 1000}{6.22 \times 1 \times 0.2} = \Delta A \times 1005 = \text{umol/L ammonia in sample}$$

   Where: 1.25 = Total volume of assay (mL)
   1000 = Conversion to liter volume
   6.22 = Millimolar extinction coefficient of NADPH at 340nm
   1 = Light path (cm)
   0.2 = Volume of the sample (mL)

   The factor 1005 multiplied by ΔA equals the ammonia concentration in the sample in umol/L.

2. Standard

   $$\frac{\text{Concentration of Standard}}{\Delta A \text{ of Standard}} \times \Delta A \text{ Sample} = \text{umol/L ammonia in Sample}$$

Sample Calculations

1. Factor
   If ΔA of sample is 0.050 then, 0.050 x 1005 = 50 umol/L ammonia in sample
2. Standard
   Concentration of ammonia standard = 500 umol/L
   ΔA of ammonia standard = 0.496
   ΔA of sample = 0.115

   $$\frac{500}{0.496} \times 0.115 = 116 \text{ umol/L ammonia in sample}$$

Quality Control

Controls are recommended to monitor the performance of the assay, providing a constant screening of the instrument, reagents and technique.

Expected Values[1]

The expected range reported for the enzymatic procedure described is 11 umol/L to 35 umol/L. It is recommended that each laboratory establish its own reference range.

Performance

1. Linearity: 600 umol/L
2. Comparison: Sixty patient samples were divided into two respective aliquots and stored in an ice bath. Analyses were performed at the same time in two separate locations using this reagent on the Cobas Fara™ and the reagent by DuPont on the DuPont ACA™. The samples ranged in ammonia concentrations form 8 umol/L to 347 umol/L. 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

References

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3. Da Fonseca-Wollheim F., J. Clin. Chem. Clin. Biochem. 11, 421, 1973.
4. Young, D.S., Effects of Pre-analytical Variables on Clinical Laboratory Tests, First Edition, AACC Press, Washington, D.C., 3.20-3.21, 1993.
5. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, Third Edition, AACC Press, Washington, D.C., 3.30-3.32, 1990.
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Rev. 3-10 P803-A7553-01