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AMMONIA REAGENT SET

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For the quantitative and enzymatic determination of ammonia in plasma.

INTRODUCTION
The level of circulating ammonia in the blood is extremely low in normal individuals due to its continuous processes of oxidative deamination and transamination of dietary and tissue amino acid. Since any appreciable level of ammonia in the blood would adversely affect the acid-base balance, a major mechanism for removal of ammonia is essential. The liver is the major organ involves in the removal of ammonia. Accordingly, plasma ammonia concentrations serve as indicator of Reye's Syndrome.[1]

PRINCIPLE
$$NH_3 + \text{2-Oxoglutarate} + NADH + H^+ \xrightarrow{GLDH} \text{L-Glutamate} + NAD$$

The ammonia reacts with 2-oxoglutarate, in the presence of L-GLDH and the coenzyme NADH, to produce L-glutamate. The resulting decrease in absorbance of NADH at 340nm is proportional to the level of ammonia in the sample.[2] Methylated amines, which interfere with other conventional procedure, do not react in the described method due to its enzyme specificity. [3]

MATERIALS PROVIDED
1. Ammonia Reagent:
   NADH 0.28mM/L, 2-Oxoglutarate 4.0mM/L, Buffer, Activators and non-reactive stabilizers.
2. L-Glutamate Dehydrogenase Solution:
   L-GLDH (beef liver >1,200 U/mL) in buffer solution
3. Ammonia Control Solution (5 µg/mL or 295 µmol/L).

MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipets to accurately measure required volumes.
2. Test tubes/rack.
3. Timer.
4. Distilled or deionized water where indicated.
5. Spectrophotometer.

WARNINGS AND PRECAUTIONS
1. For in vitro diagnostic use.
2. Avoid ingestion of reagent as toxicity has not yet been determined.
3. Serum specimens should be considered infectious and handled appropriately.
4. Use distilled or deionized water where indicated.

STORAGE AND STABILITY
The Ammonia Reagent, L-Glutamate Dehydrogenase Solution and Ammonia Control Solution must be stored at 2 - 8°C prior to reconstitution. The reagent may be used until the expiration date indicated on the package label. After reconstitution the reagent is stable for one (1) day at room temperature (18 - 25°C) and for seven (7) days when stored at 2 - 8°C. The reagent should be clean and colorless. DO NOT FREEZE L-Glutamate Dehydrogenase Solution.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity may be a sign of contamination.
2. Moisture has penetrated the vial and caking has occurred.

SPECIMEN COLLECTION AND STORAGE
Blood is collected from patients fasted at least 6 hours, using verified ammonia-free heparin as anticoagulant.[4] Donor should not clench fist during collection as muscular exertion often increases venous Ammonia levels. Since erythrocytes contain larger amounts of ammonia than plasma, hemolysis may increase results.[6] Heparin is the preferred anticoagulant because it reduces red cell ammonia production.[7] Other anticoagulants, such as sodium citrate, potassium oxalate or sodium fluoride reportedly produce spuriously high results.[8]

After drawing, blood is placed in an ice bath and plasma separated within 30 minutes. Ammonia levels in heparinized plasma are said to be stable for about 3 hours stored in an ice bath.[3] However, substantial increases in ammonia are noted after 7 hours. Stability of ammonia in heparinized blood can be extended up to 3 days if specimens are kept frozen in liquid nitrogen or in a mixture of ethanol and dry ice.[9]

Cowley et al[10] reported that venous blood is preferred to capillary blood since the latter may yield higher ammonia levels due to ammonia released through platelet activation. These authors also recommend that heparinized blood specimens be centrifuged immediately and at speeds sufficient to yield platelet poor plasma. Storage of specimens at -70°C is recommended if assays cannot be performed promptly.

INTERFERING SUBSTANCES
Inhibitors of L-GLDH include heavy metals such as silver, mercury, zinc and iron.[11] Glassware exposed to reagents containing these metals should be avoided. Plasma pyruvate levels up to 750 µg/ml do not cause interference. Certain drugs and other substances are known to influence circulating Ammonia concentrations. [12]

GENERAL INSTRUCTIONS
The reagent for Ammonia is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

PROCEDURE (AUTOMATED)
Application manual is available from us.

PROCEDURE (MANUAL)
A blank and control are included with each series of assays.
1. To Blank cuvet, add 1.0 mL of Ammonia Reagent and 0.10 mL water.
   To Control cuvet. add 1.0 mL of Ammonia Reagent and 0.10 mL Control Solution.
   To Test cuvet, add 1.0 mL of Ammonia Reagent and 0.10 mL plasma.
   Mix and wait approximately 5 minutes at 25 - 37°C for equilibration.
2. Read and record INITIAL absorbance of each cuvet at 340 nm against water.

3. Add 0.01 ml (10 µl) of L-Glutamate Dehydrogenase Solution to all cuvets. Mix by gentle inversion and wait approximately 5 minutes.

4. Read and record FINAL absorbance of each cuvets against water.

5. Calculate the absorbance change (ΔA) by subtracting FINAL absorbance from INITIAL absorbance.

6. Calculate the ammonia concentration by subtracting ΔA (Blank) from ΔA (Test) and multiplying by 30.

* USE TC - MUTI PURPOSE CALIBRATOR TO REPLACE STANDARD.

NOTE:

1. Assay may be performed by any temperature between 25 and 37°C. Temperature control is not required.
2. The procedure may be used to measure ammonia levels as low as 0.3 µg/mL(ΔA (Test) - ΔA (Blank), approximately 0.007) and as high as 20 µg/mL {A (Test) - ΔA (Blank), approximately 0.45}.

CALCULATIONS

ΔA (Blank) = INITIAL A of Blank - FINAL A of Blank
ΔA (Control) = INITIAL A of Control - FINAL A of Control
ΔA (Test) = INITIAL A of Test - FINAL A of Test

Ammonia (µg/ml) = {ΔA (Test) - ΔA (Blank)} x 30

$$\text{Factor } 30 = \frac{1.11 \times 17}{6.22 \times 0.10}$$

1.11 = Total volume of liquid in cuvet
17 = Weight (µg) of 1 µmol of ammonia
6.22 = Millimolar absorptivity of NADH at 340 nm
0.10 = Volume of specimen or sample

Note:
If a light path other than 1 cm is used, it is necessary to divide the factor 30 by the light path in cm before using it in the calculation. The calculation ignores the small change caused by addition of L-Glutamate Dehydrogenase Solution.

Example:
INITIAL A Test = 1.242 INITIAL A Blank = 1.212
FINAL A Test = 1. 125 FINAL A Blank = 1.200

ΔA (Test) = 1.242 - 1.125 = 0.117

ΔA (Blank) = 1.212 - 1.200 = 0.012

Plasma ammonia (µg/mL) = {ΔA (Test) -ΔA (Blank)} x 30 =

(0.117 - 0.012) x 30 = 0.105 x 30 = 3.15 g/mL.

To convert µg/mL to µmol/L, multiply µg/l by 59, therefore

3.15 µg/mL x 59 = 186 µmol/L.

ALTERNATE PROCEDURE

If the procedure requires a volume greater than 1.0 mL -use 3.0 ml of ammonia reagent with 0.2 mL of water, control, and sample. Use 0.02 mL (20µL) of L-glutamate instead of 0.01 mL (10 µL) for the final absorbance reading and multiply by a factor of 44.

CALIBRATION

The procedure is standardized by means of the millimolar absorptivity of NADH, which is 6.22 at 340 nm. The reductive amination of 2-oxoglutarate to form glutamate catalyzed by GLDH is coupled with the oxidation of NADH to NAD on a molar equivalent basis.

QUALITY CONTROL

It is recommended that control be included in each set of assays. Commercially available serum based control material with established ammonia 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 either reagent deterioration, instrument malfunction, or procedural errors.

LIMITATION

1. Plasma with value above 20 µg/mL should be diluted 1:1 with isotonic solution and rerun. Multiply the Final result by two (2).
2. The normal range of ammonia levels observed in serum is higher than that of plasma. Plasma ammonia concentration is affected by many commonly prescribed drugs and is well documented. [12]
3. Glassware and water should be free of ammonia.

EXPECTED VALUES

Normal values obtained by a method similar to that described in this procedure are as follows:[3]

Plasma Ammonia (µg/mL)	(µrnoI/L)
0.17 - 0.80	10 - 47

PERFORMANCE CHARACTERISTICS

1. Linearity: 0.3 µg/mL - 20 µg/mL
2. Comparison: A comparison using enzymatic procedure yielded a correlation coefficient of 0.99 with a regression equation of y = 0.97X = 0.05 (N= 41)
3. Precision studies:

Within Run (N= 20)

Mean (µg/ml)	S.D.	C.V.
4.9	0.3	6.6%
13.2	0.4	3.2%

Run to Run (N =24)

Mean (µg/ml)	S.D.	C.V.
4.8	0.4	9.6%
14.2	0.6	4.7%

4. Recovery studies: Ammonia (2.00 µg) was added to 4 sera with original ammonia levels varying from 2 - 4 µg/mL. The amount of ammonia recovered ranged from 102-105%.

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