Pariksha Biotech
A game changer in IVD

Ammonia Estimation Kit

High-Q Ammonia

Enzymatic Method

HIGH®
Command on Quality

Intended Use:
This reagent is intended for the in vitro quantitative determination of Ammonia (NH3) in human plasma

Clinical Significance
Ammonia, derived from the catabolism of amino acids and from the action of intestinal bacteria on dietary protein, is converted to urea in the liver hepatocytes and so rendered non toxic. Under normal circumstances the concentration of ammonia in the circulation remains low, typically less than 85 µg/dL. Studies have shown that excess ammonia can have a toxic effect on the central nervous system and clinical manifestations are typically neurological disturbances. Elevated levels of ammonia may be either due to:
(i) Inborn errors of metabolism; or
(ii) Secondary to other conditions.
Inborn errors of metabolism are the major cause of elevated ammonia in infants and usually the result of urea cycle enzyme deficiencies. Inherited disorders affecting the metabolism of the dibasic amino acids (lysine and ornithine) and those involving the metabolism of organic acids may also produce elevated levels of circulating ammonia. Elevated ammonia may also be observed in severe liver failure as may occur in Reye's Syndrome, viral hepatitis or cirrhosis.

Methodology:
A number of methods have been developed for the estimation of plasma ammonia and these can be broadly classified into either indirect or direct methods. In the indirect procedures, ammonia is first of all isolated, for example by the addition of alkali or the use of a cation exchange resin, after which it is measured colourimetrically by nesslerization or Berthelot reaction. These procedures are not easily automated or require dedicated equipment. Direct procedures, such as enzymatic methods, are more widely used in routine laboratories as they do not require the separation of ammonia from the specimen prior to the analytical step. Direct procedures are therefore more easily automated. The High-Q Ammonia reagent is a direct enzymatic procedure based on the following reaction sequence:-

NH4 + a-ketoglutarate + NADH
↓ *Glutamate dehydrogenase (GLDH)*
Glutamate + NAD + H2O

The reagent contains LDH in excess, to rapidly reduce endogenous pyruvate so that it does not interfere with the assay system. The High-Q Ammonia reagent also incorporates a stabilization process which renders the reagent stable in the liquid phase.

Reagent Comoposition:

Active Ingredient Concentration	
a-Ketoglutarate	7.5 mmol/L
NADH	>0.2 mmol/L
GLDH (Micro-organism)	>4000 U/L
LDH (Micro-organism)	>30,000 U/L
Tris Buffer	100 mmol/L

pH 8.7 ± 0.1 at 20°C

Reagent Preparation:
The reagent is supplied ready to use.

Stability and Storage:
When stored at 2-8°C and the reagents are properly capped they are stable until the expiration date stated on the bottle and kit box label. It is instructed that when the reagents are not in use for prolonged periods of time see that they are not exposed to the air.

Specimen Collection and Handling:
Plasma: Plasma, collected with K3-EDTA into an evacuated collection tube is recommended.

1)The blood collection tube should be completely filled with blood and immediately placed on ice.

2) Centrifuge the blood sample and separate plasma immediately. Test the plasma sample for Ammonia immediately for better results.

3) Sample must be tested for Ammonia within 30 Minutes of Blood Collection and Plasma separation. Do not test plasma after 30 Minutes as the old samples may give elevated results.

4) Samples must not be transported for Ammonia testing

System Parameters:

Reaction Type (Mode) :	Fixed Time
Reaction Direction :	Decreasing
Wave Length :	340 nm
Flow Cell Temp. :	37°C
Zero Setting with :	Distilled Water
Delay Time :	40 Seconds (A1)
Measuring Time :	90 Seconds (A2)
Reagent Volume : R1	300 µl
Reagent Volume : R2	200 µl
Calibrator / Sample Volume :	30 µl
Calibrator Concentration :	**400 µg/dL**
Linearity :	1700 µg/dL
High Normal :	150 µg/dL

Test Procedure:

Reagent	Calibrator	Plasma
Reagent-1	300 µL	300 µL
Calibrator	30 µL	——
Plasma	——	30 µL
Reagent-2	200 µL(Derive the Factor)	——
Reagent-2	——	200 µL

Mix well and immediately aspirate in to the analyzer. Record the first absorbance (A1) at 40 seconds after adding the Calibrator /Plasma. Exactly 90 Seconds after the first reading record the absorbance (A2) at 37 °C.

Calculate the change in absorbance for the Calibrator and Samples.

Calculations with calibrator:

$$\text{Ammonia (µg/dL)} = \frac{\text{A1-A2 Sample}}{\text{A1-A2 Calibrator}} \times 400 \text{ (Conc. Calibrator µg/dL)}$$

Notes:
1. The reagent and sample volumes may be altered proportionally to accommodate different analyzer requirements.2. Specimens with Ammonia concentrations greater than 1700 µg/dL. should be diluted with ammonia free water and reassayed. Multiply results by the dilution factor.

Calibration:
Calibration is required. An aqueous standard which is traceable to a primary reference standard is recommended. For calibration frequency on automated instruments refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended. Recalibration is recommended at anytime if one of the following events occurs:-
• When the lot number of reagent changes.
• When preventative maintenance is performed or a critical component is replaced.
• When the control values have shifted or are out of range and a new vial of control does not rectify the problem.

Quality Control:
To ensure adequate quality control, normal and abnormal controls with assayed values should be run as unknown samples:-
• At least every eight hours or as established by the laboratory.
• When a new bottle of reagent is used.
• After preventative maintenance is performed or a critical component is replaced. Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. The following corrective actions are recommended in such situations:-
• Repeat the same controls.
• If repeated control results are outside the limits, prepare fresh control and repeat the test.
• If results are still out of control, recalibrate with fresh standard, then repeat the test.
• If results are still out of control, perform a calibration with fresh reagent, then repeat the test.

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Limitations:
1. Hemolysed samples should not be used as erythrocytes contain levels of ammonia approximately 3 times higher than that of plasma.
2. No interference from pyruvate was observed up to a level of 0.75 mmol/L
3. No interference from ALT was observed up to a level of 4000 U/L.
4. Reliable estimations of ammonia can only be achieved if steps are taken to avoid contamination from ammonia. Sources of contamination include, but are not restricted to, cigarette smoking (patient and collection staff), laboratory atmosphere and laboratory glassware.

Expected Values:
30 - 150 µg/dL
The quoted values were derived from a normal population and should serve as a guide only. It is recommended that each Laboratory verify this range or derives a reference interval for the population that it serves

Performance Data:
The following data was obtained using the High-Q Ammonia Liquid Stable Reagents on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

Imprecision:
Imprecision was evaluated over a period of 20 days using two levels of commercial control and following the NCCLS EP5-T procedure.

Within Run:	LEVEL I	LEVEL II
Number of data points	76	76
Mean (µg/dL)	103.9	196.4
SD (µg/dL)	6.1	14.5
CV (%)	6.0	7.4

Total:	LEVEL I	LEVEL II
Number of data points	76	76
Mean (µg/dL)	103.9	196.4
SD (µg/dL)	14.8	16.7
CV (%)	14.3	8.5

Accuracy:
Comparison studies were carried out using another similar commercially available ammonia reagent. Plasma samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.
Number of sample pairs 42
Range of sample results 13 -1359 µg/dL)
Mean of reference method results 530 µg/dL)
Mean of High-Q Ammonia results 536 µg/dL)
Slope 1.002
Intercept 2.6 µg/dL)
Correlation coefficient: 0.9974

Linearity:
When run as recommended the assay is linear between 0 and 1000 µmol/L of Ammonia (0 -1700 µg/dL). Linearity on various automated instruments may vary from this value. The user should consult the specific Infinity instrument application.

Sensitivity:
When run as recommended the sensitivity of this assay is 26.0 µg/dL. (1cm light path, 340 nm).

References:
1. Clinical Chemistry Infobase: A Scientific & Management Cyclopedia. Pesce- Kaplan Publishers 1996; 2246-2320.
2. Tietz Textbook of Clinical Chemistry. Burtis CA and Ashwood ER (Eds). Second Edition, WB Saunders Company, 1994; 32:1485-88.
3. The Diagnosis of Urea Cycle Disorders, Lab Medica International, May/June 1993; 13-17.

Order Information:

Ref./Cat. No.	Pack Size	Presentation
P-AMM(E) - 12.5	12.5 ml	
P-AMM(E) - 25	25.0 ml	Two Liquid Reagents with Calibrator
P-AMM(E) - 50	50.0 ml	

Product Features

- Liquid Stable two reagents with Calibrator
- Incorporates 5 th Generation NADH Analogue
- Measuring wavelength 340 nm.
- Two Point Kinetic (Fixed Time) Assay : (40 Sec Delay+ 90 Sec Measuring)
- Linearity : 1700 µg/dL

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

Pariksha's world inside
SCAN TO EXPLORE MORE

Manufactured in India by :
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