

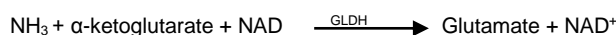
Only for in vitro use in clinical laboratory (IVD)

Clinical Significance

Ammonia (NH₃) is a reagent kit used for the quantitative determination of ammonia in plasma, based on enzymatic method using Glutamate dehydrogenase (GLDH) enzyme.

Principle

Ammonia reacts with α-ketoglutarate to form glutamate in presence of glutamate dehydrogenase. NADH is oxidized to NAD⁺ in this reaction, which is measured as decrease in absorbance at 340 nm. The rate of decrease in absorbance at 340 nm is directly proportional to the plasma ammonia concentration.



Reagents

Each Ammonia kit contains

R1 & R2: Double Reagent

Standard: 1mL

Working Reagent Preparation

R1 and R2 to be mixed in 4:1 ratio.

Stability

The reagent kit should be stored at 2° - 8°C and is stable till the expiry date indicated on the label.

Specimens

EDTA plasma or Heparinized plasma

Blood is collected from a stasis-free vein and stored in an ice bath. The plasma is then separated within 30 min. Ammonia assay should be carried out immediately. The plasma may be stored for 2 hours at 2 to 8°C.

Assay Procedure

Assay Conditions :

- Assay conditions:
 Wavelength: 340 nm
 Cuvette: 1 cm light path
 Temperature 37 °C /15-25°C

Pre warm the required amount of reagent at 25°C / 30°C before use. Perform the assay as given below:

- Adjust the instrument to zero with distilled water.
- Pipette into a Cuvette:

Reagents	Standard	Test
Working Reagent	1000 µl	1000 µl
Standard	100 µl	-
Sample	-	100 µl

- Mix & incubate 60 Sec and read initial absorbance A1 .
- Read absorbance 3 times then after, at 60 sec interval time.

Calculation

Δ OD is the average difference in absorption between the second OD and the first OD and vice versa.

$$\text{Ammonia concentration in sample (µg/dl)} = \frac{\Delta \text{Absorbance of sample}}{\Delta \text{Absorbance of standard}} \times 500$$

Reference Values

Guidance value (Ammonia) :17 - 90 µg/dl

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitations

- Anticoagulants having ammonium ions should not be used because of extreme sensitivity of the colour reaction to ammonia.
- Reaction is linear up to 1200 µg/dl. For higher values, dilute the sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.
- The working reagent is considered unsatisfactory and should not be used if the absorbance is less than 0.700 at 340 nm against distilled water.
- Do not use strongly hemolysed samples.

Quality Control

Commercial quality control for ammonia ethanol controls level 1 and level 3 are recommended for daily Quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

- Check instrument setting and light source
- Check cleanliness of all equipment in use
- Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
- Check reaction temperature.
- Check expiry date of kit and contents.

System Parameters:

Reaction type	Kinetic
Reaction Slope	Decreasing
Wavelength	340 nm
Temperature	37°C
Delay time	60 Sec
Read time	120 Sec
No. of Intervals	3
Blank	Distilled water
Sample volume	100 µl
Reagent volume, R1	800 µl
Reagent volume, R2	200 µl
Ammonia Standard Concentration	500 µg/dl
Low Normal (Ammonia)	17 µg/dl
High Normal (Ammonia)	90 µg/dl
Linearity (Ammonia)	Up to 1200 µg/dl

References

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- Howanowitz, J.H., Howanowitz, P.J., Skrodzki, C.A., Iwanski, J.A: Clin. Chem., 1984:30:906.
- Neely, W.E., Phillipson, J., Clin Chem, 1988; **34**:1868.
- Pesh-Iman, M., Kumar, S., Willis, C.E., Clin. Chem., 1978; **24**:2044.
- In-house test data. Mediclone Biotech Pvt. Ltd.

Manufactured By:

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