AMMONIA

Diagnostic reagent for determination of Ammonia concentration.
Liquid. Dual reagents. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package
ZA57	120 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of Ammonia in plasma.

GENERAL INFORMATION

Ammonia is a by-product of nitrogen metabolism and is formed predominantly through glutaminase, which is found in enterocytes in the small intestine and colon, as well as urease enzymes produced by bacteri in the intestines. The plasma ammonia concentration in the hepatic portal vein is typically five to ten times higher than in the systemic circulation. Under normal circumstances, the majority of the portal vein ammonia load is metabolized to urea in hepatocytes via the Krebs-Henseleit (urea) cycle during the first passage through the liver; this process involves intramitochondrial and cytosolic enzyme-catalyzed steps.[1]

Circulating ammonia is mainly produced from the catabolism of amino acids, either endogenous or derived from dietary proteins. The gastrointestinal tract is an important source. Plasma concentrations are usually maintained at low levels through urea synthesis and glutamine formation in the liver.[2] Renal tubular cells can also produce ammonia from glutamine and other amino acids derived from muscle and liver cells. The ammonium ion produced dissociates to some extent into ammonia and hydrogen ions depending on pH. At normal blood pH, the NH_4^+/NH_3 ratio is approximately 100 to 1. Ammonia is a gas that diffuses readily across the cell membrane into the tubular lumen, where it combines with hydrogen ions to form ammonium ions. At the acid pH of urine, the balance between NH_4^+ and NH_3 shifts strongly to the left (≈10,000 to 1), heavily supporting the formation of NH_4^+. NH_4^+ formed in the tubular lumen cannot easily cross cell membranes and is therefore retained in the tubules and excreted along with anions such as phosphate, chloride or sulfate. In normal individuals, NH_4^+ production in the tubular lumen is responsible for the excretion of ≈60% (30 to 60 mmol) of hydrogen ions. Finally, α-oxoglutarate produced in this reaction is converted to bicarbonate (up to 270 mmol/day), which helps renew bicarbonate neutralized by metabolic acid production. The amount of H^+ excreted due to NH_3 can be measured as NH_4^+. The H^+ required for NH_4^+ formation may be present in the glomerular filtrate or may be produced within the tubular cells by carbonic anhydrase-mediated synthesis of carbonic acid from CO_2. These H^+ ions are secreted into the tubular lumen via Na^+-H^+ exchangers.[3]

Ammonia enters central nervous system (CNS) tissue by passive diffusion. The rate of entry increases in proportion to plasma concentration and is pH dependent. Ammonia crosses the blood-brain barrier more easily than ammonium ion. As pH increases, the rate of entry of ammonia into CNS tissue increases as a result of the increase in ammonia over ammonium. Since the acid decomposition constant (pKa) of ammonia is 9.1 at 37 °C, about 3% of total blood ammonia is ammonia at normal physiological pH 7.4. An increase in pH to 7.6 causes an increase in ammonia to about 5% of total blood ammonia, which corresponds to a 67% increase in concentration. Animal and human studies have shown that increased ammonia concentration (hyperammonemia) produces toxic effects on the CNS.[1]

Various causes of hyperammonemia, both inherited and acquired, are known. Inherited deficiencies of urea cycle enzymes are the main cause of hyperammonemia in infants.[4] Urea cycle defects are rare disorders with an estimated average incidence of approximately 1:30,000; the most common is ornithine transcarbamylase deficiency (OTC). Some disorders of amino acid, organic acid and fatty acid metabolism may also present with hyperammonemia.[2] The two main inherited disorders are those that involve the metabolism of the dibasic amino acids lysine and ornithine and those that involve the metabolism of organic acids such as propionic acid, methylmalonic acid, isovaleric acid and others.[1] Insult to the liver, whether acute or chronic in nature, reduce its capacity to metabolize ammonia and this creates an ammonia burden on extrahepatic tissues which can result in hyperammonemia up to five times that of normal blood ammonia concentrations. Hyperammonemia is not specific to liver dysfunction and can also be observed in various other disease states, such as congenital defects in the urea cycle, Reye's syndrome and valproate poisoning.[1]

The main acquired causes of hyperammonemia are advanced liver disease and renal failure. Severe or chronic liver failure (occurring in fulminant hepatitis or cirrhosis, respectively) leads to significant disruption of normal ammonia metabolism. Since cirrhosis is accompanied by portosystemic shunting, ammonia clearance is impaired, leading to an increased concentration of ammonia in the blood.[1] Increased ammonia concentrations do not help in

the diagnosis of liver disease, prognosis or monitoring of therapy.[5]

Impaired renal function also causes hyperammonemia. As the blood urea concentration increases, more of it diffuses into the gastrointestinal tract where it is converted to ammonia. Reye syndrome, a CNS disorder with mild hepatic dysfunction, is also associated with hyperammonemia.[1] Fasting venous plasma ammonia concentration is useful in the differential diagnosis of encephalopathy when the hepatic origin of encephalopathy is uncertain.[6]It is particularly useful in the diagnosis of Reye's syndrome and hereditary disorders of urea metabolism, as well as increased ammonia concentrations due to drugs such as salicylate or valproate.[1] In acute liver injury, ammonia concentrations above 200 mmol/L (340 µg/dL) are associated with brain edema and poor prognosis.[7] However, plasma ammonia is not useful in patients with known chronic liver disease.[8]Although ammonia concentrations are higher as the degree of encephalopathy worsens, there is considerable overlap between concentrations at different stages of encephalopathy, and ammonia concentrations are increased in approximately 70% of cirrhotic patients without encephalopathy.[9] Ammonia concentrations may actually reflect better the presence of shunting blood around the portal veins than the degree of liver dysfunction.[10] The complex and synergistic relationship between ammonia, inflammation (sterile and non-sterile) and oxidative stress in the pathogenesis of hepatic encephalopathy in patients with impaired liver function is increasingly recognized.[11] In addition, clinically significant hyperammonemia may occur in neonates secondary to asphyxia, infection and sepsis.[5,13]

Ammonia is neurotoxic. Hyperammonemia has a range of presentations from an acute catastrophic illness in babies to episodes of lethargy and vomiting. Measurement of ammonia should be considered in any neonate with unexplained neurological deterioration, any older patient with unexplained encephalopathy, and children or adults with a history of episodes of vomiting and lethargy or of protein avoidance, which may indicate a mild urea-cycle defect. Increased ammonia concentration should always be confirmed in a second sample to exclude artificial increases caused by poor sample handling.[2]

TEST PRINCIPLE

UV, Enzymatic method

Ammonia combines with α-ketoglutarate and NADH in the presence of glutamate dehydrogenase (GLDH) to form glutamate and NAD^+. At 340 nm the decrease in absorbance (NADH becomes NAD^+) is proportional to the ammonia concentration in plasma.

Reaction is as follows:

NH_4^+ + 2-oxoglutarate NADH+H^+ $\xrightarrow{GDH}$ Glutamate + NAD^+ + H_2O

REAGENT COMPONENTS

Reagent 1:

α-KG	: ≤ 24.0 mmol/L
Sodium Azide	: ≤ % 0.1
TRIS	: ≤ 90 mmol/L
NADH	: ≤ 0.68 mmol/L

Reagent 2:

GLDH	: ≤ 45.0 kU/L

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[14]

SAMPLE REQUIREMENTS

Plasma collected by standardised procedure must be used. Blood should be collected from a vein without circulatory difficulties and specimen collection tubes should be tightly capped. Samples should be transported to the laboratory on ice, centrifuged preferably at 4 °C and analysed within 15 minutes of blood collection.[2]

Freezing and thawing of multiple samples should be avoided.

Ammonia activity stability in plasma:

15 minutes at +20/+25°C[12]
1 hour at +2/+8°C [2]
3 weeks at -20 °C[12]

Annotation:

Note 1: For the measurement of ammonia in blood, it is preferred to use venous samples collected without stasis and avoiding hemolysis. Capillary samples produce significantly higher results,[5,15] but can be used for rapid screening provided appropriate reference ranges are available and abnormal results are confirmed by laboratory analysis of the venous sample. Plasma ammonia concentrations are not significantly affected by diet or fasting,[5] yet some recommend a fasting state.[2]

Note 2: Plasma from samples collected on EDTA or lithium heparin is suitable for most ammonia determination methods. However, heparin may increase background absorbance with some buffers.[13,17]

Note 3: During storage of whole blood and separated plasma, the plasma ammonia concentration increases due to metabolic activity of erythrocytes and platelets and

deamination of plasma proteins and amino acids by enzymes.[18-20]
Note 4:Centrifugation should be of sufficient force to remove platelets (>2000×g). If analysis is delayed, the separated plasma can be stored at 4°C for up to 1 hour. For longer storage, freezing at -70°C is recommended.[2] Serum is not suitable because complete clotting takes longer than 15 minutes and ammonia is produced during the formation of cross-links between fibrin molecules.[22,23]

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Ammonia/Ethanol/Bicarbonate Calibrator Set.

Ammonia/Ethanol/Bicarbonate Calibrator Set (1- 2 Level)
Ref.No: ZA92

Calibration stability is 10 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Ammonia/Ethanol/Bicarbonate Conrol Set (1.- 2. Levels)
Ref.No: ELCN3

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Men	: 25 - 94 µg/dL
Women	: 19 - 82 µg/dL

Some clinical decision levels are as follows:

- **> 340 µg/dL:** Ammonia concentrations above this value are associated with brain edema and poor prognosis in acute liver injury.[7]
- **> 510 µg/dL:** Significant encephalopathy may develop.
- **> 850 µg/dL:** Coma and convulsions usually occur and are associated with hereditary metabolic diseases with neonatal onset.[2]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[25]

Unit Conversion:
µmol/L x 1.703 = µg/dL

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[26]

The determined analytic measuring interval for Ammonia is 10 – 300 µg/dL.

Detection Capability

Limit of Detection (LoD): 5 µg/dL

Limit of Quantitation (LoQ): 10 µg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[27]

Linearity

This method shows measurement linearity in the activities up to 300 µg/dL. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:10 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[28]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[29]

Repeatability (Within Run) SD (standard deviation) and CV% values of Ammonia have been given in the table 1.

Table 1. Ammonia Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
40 µg/dL	1.40	3.50	80
114 µg/dL	2.46	2.16	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[30]

Table 2. Ammonia Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
40 µg/dL	1.99	4.98	80
114 µg/dL	4.01	3.52	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[30]

Interference

Endogenous interferant and analyte concentrations that have been used in the Ammonia scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[31,32]

Interferant-Concentration	Ammonia Target(µg/dL)	N*	Observed Recovery %
Bilirubin 10 mg/dL	40	3*	94
Lipemia 500 mg/dL	40	3*	92

Hemoglobin does not interfere with ammonia determinations, although hemolyzed samples are not suitable as the concentration of ammonia in erythrocytes is approximately three times that in plasma.[20]

In addition, the following conditions should be considered in ammonia studies to avoid interference:

- Contamination of the sample with exogenous ammonia or endogenous production during sample handling and processing are the most common causes of erroneous results. Results can be affected by ammonia contamination of the atmosphere, water and laboratory equipment.[2]
- If capillary specimens are used, it is important to decontaminate the skin to avoid sweat contamination.[2]
- Smokers generally have higher plasma ammonia concentrations and it is recommended that samples be collected by a non-smoker.[2]
- In the laboratory, analysis should not be performed near a system using ammonium buffers and it should be ensured that deionized water is used and detergents are excluded.[2]
- Attention should also be paid to prevent the loss of ammonia as ammonia gas from calibrators.[2]
- Endogenous production of ammonia occurs in separated plasma due to deamination of amino acids. The glutaminase activity of γ-glutamyl transferase is an important contributor to this effect, so the ammonia concentration can be artificially increased in samples with high γ-glutamyl transferase activity.[18]
- Colonization of the laboratory analyzer with urease-positive bacteria has also been reported to produce falsely high ammonia results.[33]
- An unidentified metabolite of the antibiotic cefotaxime can interfere with the enzymatic assay for ammonia, giving a lower than zero ammonia result.[16]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[32]

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.
H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.
P264 :Wash your hands properly after using.

P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 :Wash with plenty of water and soap if it contacts with skin.

P333+P313 :Seek medical help if it irritates your skin or develops rash.

P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal

P501 :Dispose the vials and contents according to the local regulations.

REFERENCES

1. Rifai, N., Chiu, R. W., & Young, I., et al., (2023) Tietz Textbook of Laboratory Medicine (7th ed.), Chapter 51: Liver Disease, p.701-63.e12, Elsevier, St. Louis, Missouri 63043
2. Kaplan, L., Pesce, A., (2010), Kaplan: Clinical Chemistry, 5th Edition: Clinical References – Methods of Analysis, Chapter: Ammonia.
3. Rifai, N., Chiu, R. W., & Young, I., et al., (2023) Tietz Textbook of Laboratory Medicine (7th ed.), Chapter 50: Disorders of Water, Electrolytes, and Acid-Base Metabolism, p.676-700.e12, Elsevier, St. Louis, Missouri 63043
4. Burton B. Urea cycle disorders. Clin Liver Dis 2000;4:815–30.
5. Green A. When and how should we measure plasma ammonia? Ann Clin Biochem 1988; 25: 199-209.
6. Elgouhari H. What is the utility of measuring the serum ammonia level in patients with altered mental status? Cleve Clin J Med 2009;76:252–4.
7. Clemmesen J, Larsen F, Kondrup J, et al. Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. Hepatology 1999;29:648–53.
8. Arroyo V, Fernandez J, Ginès P. Pathogenesis and treatment of hepatorenal syndrome. Semin Liver Dis 2008;28:81–95.
9. Ong J, Aggarwal A, Krieger D, et al. Correlation between ammonia levels and the severity of hepatic encephalopathy. Am J Med 2003;114:188–93.
10. Aldridge DR, Tranah EJ, Shawcross DL. Pathogenesis of hepatic encephalopathy: role of ammonia and systemic inflammation. J Clin Exp Hepatol. 2015;5(Suppl 1):S7–S20. doi:10.1016/j.jceh.2014.06.004.
11. Tarantino G, Citro V, Esposito P, et al. Blood ammonia levels in liver cirrhosis: a clue for the presence of portosystemic collateral veins. BMC Gastroenterol 2009;9:21.
12. WHO Publication: Use of anticoagulants in diagnostic laboratory investigations, WHO/DIL/LAB/99.1 Rev.2:Jan 2002.
13. Losty H. Guidelines for the investigation of hyperammonaemia for inherited metabolic disorders. [cited 2007, September 25]; Available from: www.metbio.net
14. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009.
15. Cowley DM, Nagle BA, Chalmers AH, Sinton TJ. Effects of platelets on collection of specimens for assay of ammonia in plasma. Clin Chem 1985; 31: 332-333.
16. Kirk JM. Probable cefotaxime interference in enzymatic ammonia assay - a cautionary note. Ann Clin Biochem 1989; 26: 195-196
17. da Fonseca-Wollheim F, van Dam M. Interference by heparin in enzymatic determination of plasma ammonia depends on reagent composition. Clin Chem 1992; 38: 1921-1922.
18. da Fonseca-Wollheim F. Deamidation of glutamine by increased plasma gamma-glutamyltransferase is a source of rapid ammonia formation in blood and plasma specimens. Clin Chem 1990; 36: 1479-1482.
19. da Fonseca-Wollheim F. Preanalytical increase of ammonia in blood specimens from healthy subjects. Clin Chem 1990; 36: 1483-1487.
20. Howanitz JH, Howanitz PJ, Skrodzki CA, Iwanski JA. Influences of specimen processing and storage conditions on results for plasma ammonia. Clin Chem 1984; 30: 906-908.
21. Losty H. Appendix - Notes on the measurement of ammonia in blood/plasma. [cited 2007, September 25]; Available from: www.metbio.net
22. Cowley DM, Nagle BA, Chalmers AH, Sinton TJ. Effects of platelets on collection of specimens for assay of ammonia in plasma. Clin Chem 1985; 31: 332-333.
23. Mousli S, Wakid NW. Ammonia production during clot retraction and its use in assay of fibrinoligase. Clin Chem 1977; 23: 1739-1743.
24. Green A, Morgan I, Gray J. Neonatology and Clinical Biochemistry. London: ACB Venture Publications; 2003.
25. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI Document EP28-A3c. Wayne, PA: CLSI; 2010.
26. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – 1st Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018.
27. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
28. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach - 1st Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
29. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.

30. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
31. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry - First Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.
32. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry - Third Edition. CLSI Document EP07. Wayne, PA: CLSI; 2018.
33. Hayes LW, Swanson JR. Ammonia results increased by bacteria in aca needle tubing. Clin Chem 1984; 30: 1882-1883.

Archem Sağlık Sanayi ve Tic. A.Ş.
Mahmutbey Mah. Halkalı Cad. No:124 Kat:4
Bağcılar/İstanbul/Türkiye
Tel: + 90 212 444 08 92
Fax: +90 212 629 98 89
info@archem.com.tr **www.archem.com.tr**

CE

SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests