NH3L2

cobas®

Ammonia II

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058024190	08058024500	Ammonia II (300 tests)	System-ID 2094 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

20751995190	Ammonia/Ethanol/CO2 Calibrator (2 x 4 mL)	Code 20688	
20752401190	Ammonia/Ethanol/CO2 Control Normal (5 x 4 mL)	Code 20100	
20753009190	Ammonia/Ethanol/CO2 Control Abnormal (5 x 4 mL)	Code 20101	

English

System information

NH3L2: ACN 20940

Intended use

Enzymatic in vitro test for the quantitative determination of ammonia in human plasma on **cobas c** systems.

Summary

Ammonia measurements, performed with this assay in plasma are used for the diagnosis of hyperammonemia associated with various pathological conditions related to impaired ammonia metabolism (e.g. urea cycle disorders) of non-hepatic and hepatic origin (e.g. liver cirrhosis, hepatitis or hepatic encephalopathy).

Ammonia, a potent neurotoxin, is a by-product of nitrogen metabolism in the body. It is primarily formed through the action of the enzyme glutaminase in the enterocytes of the small intestine and colon, as well as by urease-producing bacteria in the gut.[1] It is transported to the liver where it is metabolized. The liver contains enzymes for the Krebs-Henseleit urea cycle, which converts ammonia into urea, a nontoxic compound that can be excreted.[2]

Ammonia levels rise when the liver is unable to metabolize it. In addition, conversion of ammonia (NH_3) to ammonium (NH_4^+) in the renal tubules occurs involving active secretion of H^+ which is a critical step in the maintenance of acid-base balance by the kidney.[3] Hyperammonemia, defined as a plasma ammonia level > 50 µmol/L (> 100 µmol/L in newborns), is highly toxic to the central nervous system and is associated with a number of clinical conditions, either in hereditary or acquired form.[4] The main acquired causes of hyperammonemia are advanced liver disease.[1,2,3,4,5,6] Hyperammonemia can lead to the development of cerebral complications including hepatic encephalopathy and cerebral edema during liver disease.[1,2,3,4,5,6]

Hyperammonemia can occur in other conditions such as inborn errors of the urea cycle,[7] Reye's syndrome[5] (primarily a central nervous system disorder), and valproate poisoning.[8] Increased ammonia formation as a compensatory mechanism in respiratory acidosis or impaired renal function may also cause hyperammonemia (as blood urea concentration increases, more diffuses into the gastrointestinal tract, where it is converted to ammonia).[9] Measurements of ammonia levels are thus critical for assessing and monitoring disease severity (especially in the case of chronic liver disease or urea cycle enzyme disorders in infants, and liver failure and hepatic encephalopathy in adults), and for treatment monitoring.[5,7,10,11] It is important to note that the interpretation of ammonia levels should be done in conjunction with other clinical findings and tests.[4,5]

Test principle

Enzymatic method, with glutamate dehydrogenase[12]

Glutamate dehydrogenase (GLDH) catalyzes the reductive amination of 2-oxoglutarate with NH_4^+ and NADPH to form glutamate and $NADP^+$.

$$NH_4^+ + \text{2-oxoglutarate} + NADPH \xrightarrow{GLDH} \text{L-glutamate} + NADP^+ + H_2O$$

The concentration of the $NADP^+$ formed is directly proportional to the ammonia concentration. It is determined by measuring the decrease in absorbance.

Reagents - working solutions

R1 BICINE[a)] buffer: 300 mmol/L, pH 8.3; GLDH (microbial): ≥ 16.7 µkat/L; detergents; preservative

R3 GLDH (microbial): ≥ 5.0 µkat/L; 2-oxoglutarate: 78 mmol/L; NADPH: ≥ 1.3 mmol/L; stabilizer; nonreactive buffer

a) BICINE = N,N-bis(2-hydroxyethyl)-glycine

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H318	Causes serious eye damage.

Prevention:

P280	Wear eye protection/ face protection.

Response:

P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.
EUH 208	Contains Glucose-6-phosphate dehydrogenase (microbial). May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	16 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
K_2- and K_3-EDTA plasma

Pay particular attention that the tubes are adequately filled according to the instruction of the tube manufacturer.

NH3L2

Ammonia II

Do not use plasma prepared with other anticoagulants.

Do not use serum since ammonia can be generated during clotting.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Smoking should be avoided prior to sampling. Tubes should be filled completely and kept tightly stoppered at all times. Place immediately on ice and centrifuge, preferably at 2-8 °C. Perform analysis within 60 minutes of venipuncture or freeze separated plasma immediately.

Ammonia concentrations can increase in vitro due to breakdown of nitrogen-containing plasma components. One known source of ammonia formation is an increased γ-glutamyltransferase activity leading to decomposition of glutamine.[13]

Avoid contamination of samples by ammonia from smoking or traffic in laboratory or patient's room, from glassware or water.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Stability in plasma:	30 min at 15-25 °C
	2 hours at 2-8 °C
	3 days at -20 °C (± 5 °C)
	at least 4 weeks at (-60)-(-90) °C

Freeze only once.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for plasma

Test definition

Reporting time	10 min		
Wavelength (sub/main)	546/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	60 µL	–	
R3	12 µL	15 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	12 µL	–	–
Decreased	6 µL	–	–
Increased	12 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O It is highly recommended to always use fresh water from closed vessels. S2: Ammonia/Ethanol/CO2 Calibrator
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - automatically every 2 weeks - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against a primary standard.

Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 16 weeks.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

cobas c systems automatically calculate the analyte concentration of each sample in the unit µmol/L (µg/dL).

Conversion factor: µmol/L × 1.703 = µg/dL

Limitations - interference

Criterion: Recovery within ± 5 µmol/L of initial values of samples ≤ 50 µmol/L and within ± 10 % for samples > 50 µmol/L

Icterus:[14] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[14] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62.1 µmol/L or 100 mg/dL). Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal plasma. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[14] No significant interference up to an L index of 700. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.

Exceptions: Cefoxitin and Intralipid cause artificially high ammonia results at the therapeutic drug level.[15,16]

Physiological plasma concentrations of sulfasalazine may lead to false results.

Temozolomide at therapeutic concentrations may lead to erroneous results.

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[17]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All

special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

10-1000 µmol/L (17-1703 µg/dL)

Determine samples having higher concentrations via the rerun function. For samples with higher concentrations, the rerun function decreases the sample volume by a factor of 2. The results are automatically multiplied by this factor.
Please consider the recommended sample stability.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 10 µmol/L (17 µg/dL)
Limit of Detection	= 10 µmol/L (17 µg/dL)
Limit of Quantitation	= 10 µmol/L (17 µg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a precision coefficient of variation of ≤ 20 %. It has been determined using low concentration ammonia samples.

Expected values

µmol/L

EDTA plasma[18]

Women	11-51 µmol/L
Men	16-60 µmol/L

µg/dL*

EDTA plasma[18]

Women	18.7-86.9 µg/dL
Men	27.2-102 µg/dL

*calculated by unit conversion factor

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

Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean* µmol/L	*SD* µmol/L	*CV* %
AEC Control N[b)]	63.3	0.767	1.2
AEC Control A[c)]	230	1.82	0.8
Human plasma 1	28.1	0.818	2.9
Human plasma 2	61.6	0.663	1.1
Human plasma 3	96.1	0.937	1.0
Human plasma 4	511	1.70	0.3
Human plasma 5	944	17.8	1.9

Intermediate precision	*Mean* µmol/L	*SD* µmol/L	*CV* %
AEC Control N[b)]	63.3	1.06	1.7
AEC Control A[c)]	230	2.59	1.1
Human plasma 1	28.1	0.984	3.5
Human plasma 2	61.6	0.954	1.5
Human plasma 3	96.1	1.31	1.4
Human plasma 4	511	3.27	0.6
Human plasma 5	944	18.1	1.9

b) Ammonia/Ethanol/CO2 Control Normal

c) Ammonia/Ethanol/CO2 Control Abnormal

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Ammonia values for human plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 70

Passing/Bablok[19]	Linear regression
y = 0.995x + 2.20 µmol/L	y = 0.996x + 0.203 µmol/L
τ = 0.977	r = 1.000

The sample concentrations were between 12.9 and 983 µmol/L.

Ammonia values for human plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 64

Passing/Bablok[19]	Linear regression
y = 0.975x + 6.35 µmol/L	y = 0.975x + 5.20 µmol/L
τ = 0.988	r = 1.000

The sample concentrations were between 13.0 and 996 µmol/L.

Ammonia values for human plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 75

Passing/Bablok[19]	Linear regression
y = 1.000x - 4.00 µmol/L	y = 0.993x - 1.15 µmol/L
τ = 0.988	r = 1.000

The sample concentrations were between 10.2 and 983 µmol/L.

References

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606