NH3L

cobas®

Ammonia

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20766682 322	Ammonia 150 tests	System-ID 07 6668 2	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
20751995 190	Ammonia/Ethanol/CO2 Calibrator (2 x 4 mL)	Code 688	
20752401 190	Ammonia/Ethanol/CO2 Control Normal (5 x 4 mL)	Code 100	
20753009 190	Ammonia/Ethanol/CO2 Control Abnormal (5 x 4 mL)	Code 101	

English

System information

For **cobas c** 311/501 analyzers:

NH3L: ACN 478

For **cobas c** 502 analyzer:

NH3L: ACN 8478

Intended use

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

Summary[1]

Ammonia is generated primarily in the gastrointestinal tract by metabolism of nitrogenous compounds. An excess of ammonia can be toxic to the central nervous system. The Krebs-Henseleit urea cycle provides a means of disposal of ammonia by metabolizing ammonia to urea in the liver.

Hyperammonemia in infants can be caused by inherited deficiencies of the urea cycle enzymes or acquired through acute (as in Reye's syndrome) or chronic (as in cirrhosis) liver disease. In adults, elevated ammonia levels can aid in diagnosis of liver failure or hepatic encephalopathy from advanced liver diseases such as viral hepatitis or cirrhosis.

Test principle

Enzymatic method, with glutamate dehydrogenase.[2,3]

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: 330 mmol/L, pH 8.3; GLDH (microbial): ≥ 234 µkat/L; 2-oxoglutarate: 50 mmol/L; detergent; preservative; nonreactive stabilizer

R3 NADPH: ≥ 1.0 mmol/L; preservative; nonreactive buffer

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

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

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Reagent handling

Unscrew only the grey coloured R3 screw cap using the Open/Close tool (GMMI no. 04857933-190). Keep the screw cap. Prevent it from any contamination. Place the reagent cassette in an environment which is free from cigarette smoke, NH3 containing cleaners, and exhaust fumes of any kind. During storage of the cassette at room temperature ensure that it is securely stored so that it doesn't fall over. The cassette must not be covered during the storage at room temperature. Prevent the cassette from debris of any kind. Protect open cassette from sun light, do not store it at the window. After 24 h storage, re-screw the same grey screw cap on the R3 bottle. Mix cassette gently avoiding foam. Load the cassette into the instrument.

Storage and stability

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Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 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-EDTA plasma (free from hemolysis and lipemia)

IMPORTANT

Do not use plasma prepared with other anticoagulants.

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

Collect blood from stasis-free vein of fasting patient. 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 4 °C. Perform analysis within 20 to 30 minutes of venipuncture.

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

Ammonia concentrations can increase in vitro due to breakdown of nitrogen-containing plasma components. One known source of spontaneous ammonia formation is an increased γ-glutamyltransferase activity leading to decomposition of glutamine.[3]

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.

Centrifuge samples containing precipitates before performing the assay.

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

cobas c 311 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 25-57	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Decrease	
Units	µmol/L (µg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	40 µL	32 µL

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R3	20 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	20 µL	–	–
Decreased	10 µL	–	–
Increased	20 µL	–	–

cobas c 501/502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 36-70		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	µmol/L (µg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	40 µL	32 µL	
R3	20 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	20 µL	–	–
Decreased	10 µL	–	–
Increased	20 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2: Ammonia/Ethanol/CO2 Calibrator
Calibration mode	Linear
Calibration frequency	2-point calibration
	- after **cobas c** pack change
	- blank re-calibration automatically every 3 days
	- as required following quality control procedures

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. 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

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

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

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at an ammonia concentration of 50 µmol/L (85 µg/dL).

Icterus:[4] No significant interference up to an I index of 10 for conjugated and 30 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 171 µmol/L (10 mg/dL) and approximate unconjugated bilirubin concentration: 513 µmol/L or 30 mg/dL).

Hemolysis:[4] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124.2 µmol/L or 200 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 (native):[4] No significant interference up to an L index of 50. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

γ-Globulin: γ-Globulin significantly increases the apparent ammonia concentration when 3 g/dL are added to a human plasma pool.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[5,6]

Exceptions: Cefoxitin and Intralipid cause artificially high and low ammonia results respectively at the therapeutic drug level.

Physiological plasma concentrations of Sulfasalazine and Sulfapyridine may lead to false results. Temozolomide at therapeutic concentrations may lead to erroneous results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[7]

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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

10-700 µmol/L (17-1192 µg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Lower detection limit of the test

10 µmol/L (17 µg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

EDTA plasma[8]

Females	11-51 µmol/L	(18.7-86.9 µg/dL)
Males	16-60 µmol/L	(27.2-102 µg/dL)

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. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*

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AEC Control N	60.7 (103)	1.4 (2)	2.3
AEC Control A	202 (344)	2 (3)	0.8
Human plasma 1	28.6 (48.7)	2.5 (4.3)	8.8
Human plasma 2	585 (996)	1 (2)	0.2
Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L (µg/dL)	*µmol/L (µg/dL)*	*%*
AEC Control N	56.9 (97.1)	2.8 (4.8)	4.9
AEC Control A	203 (346)	4 (7)	1.8
AEC Control N 1:2 dil.	28.1 (47.7)	2.6 (4.4)	9.4
AEC Calibrator	318 (542)	5 (9)	1.5

Method comparison

Ammonia values for human plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined on Roche/Hitachi 917/MODULAR P analyzers (x), using the corresponding Roche/Hitachi reagent.

Sample size (n) = 171

Passing/Bablok[9]	Linear regression
y = 0.996x + 5.11 µmol/L	y = 1.007x + 4.06 µmol/L
τ = 0.970	r = 0.999

The sample concentrations were between 18.1 and 444 µmol/L (30.8 and 756 µg/dL).

References

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9 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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.

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 after reconstitution or mixing

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