NH3L2

cobas®

Ammonia II

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
07229593 190	Ammonia II (150 tests)	System-ID 07 7606 8	Roche/Hitachi **cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

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:

NH3L2: ACN 479

For **cobas c** 502 analyzer:

NH3L2: ACN 8479

Intended use

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

Summary

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.[1]

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.[1]

Test principle

Enzymatic method, with glutamate dehydrogenase[2]

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.

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.

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.[3]

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

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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 / 24-57		
Wavelength (sub/main)	546/340 nm		
Reaction direction	Decrease		
Units	µmol/L (µg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	85 µL	-	
R3	17 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	17 µL	–	–
Decreased	8.5 µL	–	–
Increased	17 µL	–	–

cobas c 501/502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 36-70		
Wavelength (sub/main)	546/340 nm		
Reaction direction	Decrease		
Units	µmol/L (µg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	85 µL	-	
R3	17 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	17 µL	–	–
Decreased	8.5 µL	–	–
Increased	17 µL	–	–

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	2-point 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. 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 × 1.703 = µg/dL

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at an ammonia concentration of 50 µmol/L.

Icterus:[4] 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:[4] 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):[4] 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.[5,6]

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.[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-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 required in certain cases.

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

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.

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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

EDTA plasma[8]

Women	11-51 µmol/L	(18.7-86.9 µg/dL)
Men	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

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5-A3 requirements (2 aliquots per run, 2 run per day, 21 days). The following results were obtained:

Repeatability	*Mean µmol/L (µg/dL)*	*SD µmol/L (µg/dL)*	*CV %*
AEC Control N[b)]	66.6 (113)	1.40 (2.38)	2.1
AEC Control A[c)]	243 (414)	3.45 (5.88)	1.4
Human plasma 1	26.0 (44.3)	1.26 (2.15)	4.8
Human plasma 2	57.7 (98.3)	1.63 (2.78)	2.8
Human plasma 3	110 (187)	1.62 (2.76)	1.5
Human plasma 4	492 (838)	4.12 (7.02)	0.8
Human plasma 5	863 (1470)	9.54 (16.2)	1.1
Intermediate precision	*Mean µmol/L (µg/dL)*	*SD µmol/L (µg/dL)*	*CV %*
AEC Control N[b)]	67.9 (116)	1.61 (2.74)	2.4
AEC Control A[c)]	243 (414)	4.26 (7.25)	1.8
Human plasma 1	26.0 (44.3)	1.29 (2.20)	4.9
Human plasma 2	57.7 (98.3)	1.72 (2.93)	3.0
Human plasma 3	110 (187)	1.92 (3.27)	1.7
Human plasma 4	480 (817)	6.30 (10.7)	1.3
Human plasma 5	853 (1453)	12.4 (21.1)	1.5

b) Ammonia/Ethanol/CO2 Control Normal

c) Ammonia/Ethanol/CO2 Control Abnormal

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

Ammonia values for human plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the AMM reagent of Beckman Coulter on a Beckman Synchron DxC 800 analyzer (x).

Sample size (n) = 111

Passing/Bablok[9]	Linear regression
y = 1.002x – 2.01 µmol/L	y = 1.020x – 4.88 µmol/L
τ = 0.979	r = 1.000

The sample concentrations were between 17.0 and 984 µmol/L (29.0 and 1676 µg/dL).

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

References

1 Balistreri WF, Rej R. Liver function. In: Burtis CA, Ashwood ER, eds. Tietz Fundamentals of Clinical Chemistry. 4th ed.Philadelphia: WB Saunders 1996;539-568.

2 Van Anken HC, Schiphorst ME. A kinetic determination of ammonia in plasma. Clin Chim Acta 1974;56:151-157.

3 Da Fonseca-Wollheim F. Deamidation of glutamine by increased plasma γ-glutamyltransferase is a source of rapid ammonia formation in blood and plasma specimens. Clin Chem 1990;36:1479-1482.

4 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

5 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

6 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

7 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

8 Da Fonseca-Wollheim F. Direkte Plasmaammoniakbestimmung ohne Enteiweissung. Z Klin Chem Klin Biochem 1973;11:426-431.

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.

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 (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

0107229593190c501V2.0

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CE 0123

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