

NH3L

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 COBAS INTEGRA 400 plus COBAS INTEGRA 800
20751995 190	Ammonia/Ethanol/CO ₂ Calibrator (2 × 4 mL)	System-ID 07 5199 5
20752401 190	Ammonia/Ethanol/CO ₂ Control Normal (5 × 4 mL)	System-ID 07 5240 1
20753009 190	Ammonia/Ethanol/CO ₂ Control Abnormal (5 × 4 mL)	System-ID 07 5300 9

English

System information

Test NH3L, test ID 0-168

Intended use

In vitro test for the quantitative determination of the ammonia concentration in human plasma on COBAS INTEGRA systems.

Summary¹

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

Glutamate dehydrogenase (GLDH) catalyzes the reductive amination of 2-oxoglutarate with NH₄⁺ and NADPH to form glutamate and NADP⁺.



The concentration of the NADP⁺ formed is directly proportional to the ammonia concentration. It is determined by measuring the decrease in absorbance at 340 nm.

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

R2 NADPH: ≥ 1.0 mmol/L; preservative; nonreactive buffer

a) N,N-bis[2-hydroxyethyl]glycine

R1 is in position A and R2 is in position B.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on cobas c pack label
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	12 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	12 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. EDTA plasma

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.

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 20-30 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 at storage higher than -38 °C 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, glassware, or water.

Centrifuge samples containing precipitates before performing the assay.

Stability in plasma: 3 weeks at -38 °C³

Materials provided

See "Reagents – working solutions" section for reagents.

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.

Application for plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-R2-S
Reaction direction	Decrease
Wavelength A/B	340/629 nm
Calc. first/last	T ₀ /58
Unit	µmol/L

Pipetting parameters

		Diluent (H ₂ O)
R1	40 µL	10 µL
R2	20 µL	12 µL
Sample	20 µL	30 µL
Total volume	132 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-R2-S
Reaction direction	Decrease

Wavelength A/B	340/629 nm
Calc. first/last	T ₀ /60
Unit	μmol/L

Pipetting parameters

		Diluent (H ₂ O)
R1	40 μL	10 μL
R2	20 μL	12 μL
Sample	20 μL	30 μL
Total volume	132 μL	

Calibration

Calibrator	Ammonia/Ethanol/CO ₂ Calibrator Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

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

Quality control

Quality control	Ammonia/Ethanol/CO ₂ Control Normal Ammonia/Ethanol/CO ₂ Control Abnormal
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

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

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Icterus:⁴ In samples with normal ammonia concentrations, no significant interference was found up to an I index of 11 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 188 μmol/L or 11 mg/dL).

In samples with pathological ammonia concentrations, no significant interference was found up to a bilirubin level of 906 μmol/L or 53 mg/dL.

Hemolysis:⁴ No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62 μmol/L or 100 mg/dL).

Lipemia (Intralipid):⁴ No significant interference up to an L index of 80. There is 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.

Anticoagulants: Fluoride, citrate and heparin must not be used.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{5,6} Exceptions: Sodium cefoxitin causes artificially high ammonia values at the tested drug level. Physiological plasma concentrations of Sulfasalazine or 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 COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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)

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 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 30).

Expected values

EDTA plasma⁸

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 COBAS INTEGRA 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 and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

	Level 1	Level 2
Mean	60.2 μmol/L (103 μg/dL)	231 μmol/L (393 μg/dL)
CV repeatability	2.2 %	0.9 %
CV intermediate precision	3.6 %	1.8 %

Method comparison

Ammonia values for human plasma samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Ammonia reagent (y) were compared with those determined using a commercially available reagent for ammonia on a COBAS INTEGRA 700 analyzer (x). Samples were measured in duplicate.

COBAS INTEGRA 700 analyzer		
Sample size	(n)	114
Correlation coefficient	(r)	0.996
	(r _s)	0.982
Linear regression	y = 1.10x + 7.5 μmol/L	
Passing/Bablok ⁹	y = 1.10x + 5.0 μmol/L	

The sample concentrations were between 0 and 580 μmol/L (0 and 988 μg/dL).




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.

Symbols

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

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

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