ETOH2

cobas®

Ethanol Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057630190	08057630500	Ethanol Gen.2 (850 tests)	System-ID 2056 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08445672190	08057630500	Ethanol Gen.2 (150 tests)	System-ID 2056 002	**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

ETOH2: ACN 20560 (Serum/plasma)

ETOH2U: ACN 20561 (Urine)

Intended use

In vitro test for the quantitative determination of ethanol in human serum, plasma and urine on **cobas c** systems.

Summary

Detection of ethanol in human serum, plasma and urine with this assay is used as an aid in the diagnosis and treatment of alcohol intoxication or poisoning in individuals with suspected exposure.

Ethanol (EtOH) is the most widely used and often abused addictive substance and therefore ethyl alcohol determinations are among the most frequent analyses required in the forensic and clinical toxicology laboratory.[1] Alcohol consumption is a causal factor in more than 60 types of diseases and injuries resulting in approximately 2.5 million deaths worldwide each year.[2]

After ingestion, ethanol is absorbed by the oral, gastric and small intestinal mucosa. Detoxification of EtOH starts in the stomach and is facilitated by alcohol dehydrogenase (ADH). The majority of EtOH is metabolized in the liver to acetaldehyde by ADH, the catalase and the microsomal ethanol oxidizing system. Acetaldehyde is further metabolized to acetic acid via acetaldehyde dehydrogenase.[3] A part of the remaining ingested ethanol undergoes non-oxidative metabolism resulting in the following metabolites: ethyl glucuronide (EtG), ethyl sulfate (EtS), fatty acid ethyl esters (FAEEs) and phosphatidylethanol (PEth).[4,5] The elimination rate varies among individuals and is influenced by drinking practices (heavy drinkers have an increased elimination rate due to enzyme induction).[1]

Compared to other alcohol biomarkers ethyl glucuronide (EtG)/Ethyl sulphate (EtS) or phosphatidylethanols (PEth), ethanol has a short window of detection and is therefore useful for detection of recent alcohol consumption and alcohol intoxication.[6]

Test principle

Enzymatic method with alcohol dehydrogenase.

Ethyl alcohol and NAD are converted to acetaldehyde and NADH by ADH.

$$\text{Ethyl alcohol} + NAD^+ \xrightarrow{\text{ADH}} \text{acetaldehyde} + NADH + H^+$$

The NADH formed during the reaction, measured photometrically as a rate of change in absorbance, is directly proportional to the ethyl alcohol concentration.

Reagents - working solutions

R1 Buffer; preservatives

R3 NAD (yeast): ≥ 3 mmol/L; ADH (EC 1.1.1.1; yeast; 25 °C): ≥ 617 µkat/L (37 U/mL); stabilizers; preservatives

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.

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:	12 weeks

Specimen collection and preparation[7,8]

Do not use alcohol or other volatile disinfectants at the site of venipuncture. Aqueous Zephiran (benzalkonium chloride), aqueous Merthiolate (thimerosal), or povidone-iodine may be used.

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

Only the specimens listed below were tested and found acceptable.
Serum, Plasma: Li-heparin and K_2-EDTA

Stability:[9]	2 days at 20 °C (± 5 °C)
	2 weeks at 2-8 °C
	4 weeks at -20 °C (± 5 °C)

Freeze only once.

Plasma: NaF/Na_2-EDTA and NaF/K-oxalate

Stability:[9]	2 weeks at 20 °C (± 5 °C)
	3 months at 2-8 °C
	6 months at -20 °C (± 5 °C)

Freeze only once.

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.

Urine: Use random urine.

Stability:[10]	30 days at 2-8 °C

Freeze only once.

Storage: Samples must be tightly closed.

Centrifuge samples containing precipitates before performing the assay.

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

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Each laboratory should establish guidelines for determining acceptability of specimens and the corrective action to be taken if a specimen is considered unacceptable.

With respect to specimens procured for medicolegal purposes, each legal jurisdiction may have specific requirements concerning the collection and storage of specimens from living subjects, which should be followed as rigorously as possible.[11]

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.

Repeat assays must be performed on freshly poured cups, due to evaporation of alcohol.

When using Ammonia/Ethanol/CO2 Calibrator: Do not leave calibrator cups open for longer than 30 minutes at 15-25 °C.

When using Ammonia/Ethanol/CO2 Controls: Do not leave control cups open for longer than 1 hour at 15-25 °C.

Application for serum, plasma and urine

Test definition

Reporting time	10 min	
Wavelength (sub/main)	700/340 nm	
Reagent pipetting		Diluent (H_2O)
R1	40 µL	–
R3	40 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	3.2 µL	–	–
Decreased	1.6 µL	–	–
Increased	3.2 µL	–	–

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

Calibration

Application for serum/plasma (ACN 20560)

Calibrators	S1: H_2O
	S2: Ammonia/Ethanol/CO2 Calibrator
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - every 6 weeks on-board - as required following quality control procedures

Application for urine (ACN 20561)

Transfer of calibration from serum/plasma application (ACN 20560)

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

Traceability: This method has been standardized against NIST-traceable materials.

Quality control

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

Serum/plasma:	Ammonia/Ethanol/CO2 Control Normal, Ammonia/Ethanol/CO2 Control Abnormal
Urine:	Ammonia/Ethanol/CO2 Control Normal, Ammonia/Ethanol/CO2 Control Abnormal

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[12]

cobas c systems automatically calculate the analyte concentration of each sample in the unit mmol/L (g/L, mg/dL, ‰).

Conversion factors:	mmol/L x 0.04608 = g/L
	mmol/L x 4.608 = mg/dL
	mmol/L x 0.0374 = ‰

Limitations - interference

Criterion: Recovery within ± 2.2 mmol/L of initial values of samples ≤ 21.7 mmol/L and within ± 10 % for samples > 21.7 mmol/L.

Serum/plasma

Icterus:[13] No significant interference up to an I index of 30 for conjugated bilirubin and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 513 µmol/L or 30 mg/dL; approximate unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[13] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124.2 µmol/L or 200 mg/dL).

Lipemia (Intralipid):[13] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

LDH/lactic acid (using a dose-response curve with purified LDH fractions added to a 30 mmol/L lactic acid solution): No significant interference up to 2000 U/L LDH.

Urine

Hemolysis: No significant interference up to an H index of 750 (approximate hemoglobin concentration: 466 µmol/L or 750 mg/dL).

Glucose: No significant interference from glucose up to a concentration of 111 mmol/L (2000 mg/dL).

Urea: No significant interference from urea up to a concentration of 1800 mmol/L (10811 mg/dL).

Creatinine: No significant interference from creatinine up to a concentration of 22.1 mmol/L (250 mg/dL).
CAUTION: Urine containing sugars and contaminated with microorganisms may yield a false positive result due to fermentation of sugar to alcohol.
CAUTION: Do not use volatile solvents in the work area when performing assays. Do not perform sample preparation (especially spiking of pools) in the immediate work area. Vapor contamination of reagents can impact calibration stability.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[15]

Serum/plasma/urine

The **cobas c** Ethanol Gen.2 reagent is specific for ethanol. The following cross-reactants were measured at 2000 mg/dL:

Compound	**% Cross-reactivity (serum)**	**% Cross-reactivity (urine)**
n-Propanol	8.0	9.9
n-Butanol	2.8	1.5
Isopropanol	0.2	0.5

Acetone	0.0	0.2
Ethylene glycol	0.0	0.2
Methanol	-0.1	0.2
Acetaldehyde	-1.1	-0.3

$$\frac{\text{mg/dL apparent ethanol}}{\text{mg/dL cross-reactant in sample}} \times 100 = \%\ \text{cross-reactivity}$$

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

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

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

Serum, plasma and urine

2.2-108 mmol/L (0.101-4.98 g/L)

Specimen dilution

NOTE: Do not use automatic rerun.

Determine samples with higher concentrations via the decreased sample volume function. Use a fresh aliquot from a sample stored tightly closed. Reduction of sample volume via the decreased sample volume function represents a 1:2 dilution. These results are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 2.2 mmol/L (0.101 g/L)
Limit of Detection	= 2.2 mmol/L (0.101 g/L)
Limit of Quantitation	= 2.2 mmol/L (0.101 g/L)

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 total error of 20 %. It has been determined using low concentration ethanol samples.

Expected values

Serum/plasma[12]

mmol/L

10.9-21.7 mmol/L	Flushing, slowing of reflexes, impaired visual acuity
> 21.7 mmol/L	Depression of CNS
> 86.8 mmol/L	Fatalities reported

g/L*

* calculated by unit conversion factor

0.5-1 g/L	Flushing, slowing of reflexes, impaired visual acuity
> 1 g/L	Depression of CNS
> 4 g/L	Fatalities reported

Urine

The ratio of the urinary ethanol concentration to blood ethanol concentration is often reported as 1.3:1. However other lower or higher ratios might be used depending on the patient population and related factors such as the volume of urine that is produced and excreted.[17]

The legal definition of intoxication varies according to local law. Each laboratory should establish an acceptable reporting format and identify procedures for the reporting of abnormal results. Clinical consideration and professional judgment should be applied to the interpretation of any alcohol test results.

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.

Serum/plasma

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
AEC-N[a)]	10.8	0.125	1.2
AEC-A[b)]	31.8	0.300	0.9
Human serum 1	5.07	0.160	3.2
Human serum 2	16.4	0.146	0.9
Human serum 3	21.0	0.211	1.0
Human serum 4	52.7	0.271	0.5
Human serum 5	91.8	0.562	0.6
Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
AEC-N[a)]	10.8	0.181	1.7
AEC-A[b)]	31.8	0.425	1.3
Human serum 1	5.07	0.194	3.8
Human serum 2	16.4	0.234	1.4
Human serum 3	21.0	0.315	1.5
Human serum 4	52.0	0.622	1.2
Human serum 5	91.8	1.13	1.2

Urine

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
AEC-N[a)]	10.8	0.138	1.3
AEC-A[b)]	31.5	0.198	0.6
Human urine 1	5.34	0.0981	1.8
Human urine 2	16.1	0.136	0.8
Human urine 3	23.0	0.169	0.7

Human urine 4	54.6	0.305	0.6
Human urine 5	92.6	0.487	0.5
Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
AEC-N[a)]	10.8	0.184	1.7
AEC-A[b)]	31.8	0.404	1.3
Human urine 1	5.43	0.124	2.3
Human urine 2	16.1	0.370	2.3
Human urine 3	23.0	0.302	1.3
Human urine 4	54.6	0.687	1.3
Human urine 5	91.8	2.67	2.9

a) Ammonia/Ethanol/CO2 Control Normal

b) 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

Ethanol values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 73

Passing/Bablok[18]	Linear regression
y = 1.018x - 0.218 mmol/L	y = 1.011x - 0.175 mmol/L
τ = 0.968	r = 0.998

The sample concentrations were between 2.60 and 101 mmol/L.

Urine

Sample size (n) = 72

Passing/Bablok[18]	Linear regression
y = 1.026x - 0.112 mmol/L	y = 1.022x - 0.0444 mmol/L
τ = 0.985	r = 1.000

The sample concentrations were between 3.40 and 102 mmol/L.

Ethanol values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 67

Passing/Bablok[18]	Linear regression
y = 1.022x + 0.129 mmol/L	y = 1.020x - 0.205 mmol/L
τ = 0.969	r = 0.999

The sample concentrations were between 2.30 and 104 mmol/L.

Urine

Sample size (n) = 68

Passing/Bablok[18]	Linear regression
y = 1.003x - 0.275 mmol/L	y = 1.013x - 0.628 mmol/L
τ = 0.976	r = 0.999

The sample concentrations were between 2.50 and 103 mmol/L.

Ethanol values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 75

Passing/Bablok[18]	Linear regression
y = 1.017x + 0.00334 mmol/L	y = 1.017x - 0.0168 mmol/L
τ = 0.978	r = 1.000

The sample concentrations were between 5.93 and 104 mmol/L.

Urine

Sample size (n) = 75

Passing/Bablok[18]	Linear regression
y = 1.006x + 0.0109 mmol/L	y = 1.005x + 0.0511 mmol/L
τ = 0.993	r = 1.000

The sample concentrations were between 6.11 and 102 mmol/L.

References

1 Langman LJ, Bechtel LK, Holstege CP. Clinical Toxicology. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 43, p. 454-454.e84.

2 Maenhout TM, De Buyzere ML, Delanghe JR. Non-oxidative ethanol metabolites as a measure of alcohol intake. Clin Chim Acta 2013 Jan 16;415:322-329.

3 Staufer K, Yegles M. Biomarkers for detection of alcohol consumption in liver transplantation. World J Gastroenterol 2016 Apr 14;22(14):3725-3734.

4 Kummer N, Lambert WE, Samyn N, Stove CP. Alternative sampling strategies for the assessment of alcohol intake of living persons. Clin Biochem 2016 Sep;49(13-14):1078-1091.

5 Oppolzer D, Barroso M, Gallardo E. Bioanalytical procedures and developments in the determination of alcohol biomarkers in biological specimens. Bioanalysis 2016 Feb;8(3):229-251.

6 Topic A, Djukic M. Diagnostic characteristics and application of alcohol biomarkers. Clin Lab 2013;59(3-4):233-245.

7 Proposed guidelines NCCLS: Blood Alcohol Testing in the Clinical Laboratory. NCCLS Vol. 8 No. 10. December 1988.

8 Tietz NW. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunder 1987;890.

9 Tietz NW, ed. Clinical Guide to Laboratory Tests, 4th ed. Philadelphia, PA: WB Saunders Company 2006;1344-1346.

10 Levine B, Smith ML. Stability of drugs of abuse in biological specimens. Forensic Sci Rev 1990;2:148-156.

11 Garriott JC, ed. Medicolegal Aspects of Alcohol. 3rd ed. Tucson, AZ: Lawyers & Judges Publishing Co, Inc 1996:262.

12 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;224-225.

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

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

15 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.

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

17 Jones AW. Reference Limits for Urine/Blood Ratios of Ethanol in Two Successive Voids from Drinking Drivers. J Anal Toxicol 2002;26(Sep):333-339.

18 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:

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

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