

Ethanol Gen.2**Order information**

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

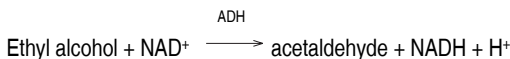
Ethyl alcohol determinations are among the most frequent analyses required in the forensic and clinical toxicology laboratory. Ethyl alcohol measurements are used in the diagnosis and treatment of alcohol intoxication and poisoning.

Early techniques for blood alcohol determination used distillation, aeration, or diffusion to separate the alcohol from the plasma matrix. The distilled alcohol was then measured by oxidation of the alcohol by strong oxidizing agents. However, these methods lacked specificity, since other oxidizable compounds could also be distilled into and react in the reaction mixture.¹ While there are many acceptable published procedures, including gas chromatographic and osmometric methods, the enzymatic technique described below, based on the information given by Bucher and Redetzki², is specific and simple to perform.

Test principle

Enzymatic method with alcohol dehydrogenase.

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



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 $\mu\text{kat/L}$ (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^{3,4}

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 K₂-EDTA

Stability:⁵

- 2 days at 25 °C
- 2 weeks at 5 °C
- 4 weeks at -15 °C

Plasma: NaF/Na₂-EDTA and NaF/K-oxalate

Stability:⁵

- 2 weeks at 25 °C
- 3 months at 5 °C
- 6 months at -15 °C

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:⁶ 30 days at 4 °C

Storage: Samples must be tightly closed.

Specimen should not be repeatedly frozen and thawed (only 1 freeze and thaw cycle is allowed).

Centrifuge samples containing precipitates before performing the assay.

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

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.⁷

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 ₂ O)
R1	40 µL –
R3	40 µL –

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (H ₂ O)
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 ₂ O S2: Ammonia/Ethanol/CO2 Calibrator
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - after 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:	Quantitative urine controls are recommended for routine quality control.

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 12 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.

Calculation⁸

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 ±10 % of initial value at an ethanol concentration of 21.7 mmol/L (1 g/L, 100 mg/dL).

Serum/plasma

Icterus:⁹ 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:⁹ No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124.2 µmol/L or 200 mg/dL).

Lipemia (Intralipid):⁹ 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.^{10,11}

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.¹²

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.¹¹

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.

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 $n \geq 60$ 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*⁸

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.¹³

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

<i>Repeatability</i>	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>mmol/L</i>	<i>mmol/L</i>	<i>%</i>
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

	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>mmol/L</i>	<i>mmol/L</i>	<i>%</i>
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

<i>Repeatability</i>	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>mmol/L</i>	<i>mmol/L</i>	<i>%</i>
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

	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>mmol/L</i>	<i>mmol/L</i>	<i>%</i>
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 ¹⁴	Linear regression
$y = 1.018x - 0.218$ mmol/L	$y = 1.011x - 0.175$ mmol/L
$\tau = 0.968$	$r = 0.998$

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

Urine

Sample size (n) = 72

Passing/Bablok ¹⁴	Linear regression
$y = 1.026x - 0.112$ mmol/L	$y = 1.022x - 0.0444$ mmol/L
$\tau = 0.985$	$r = 1.000$

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 ¹⁴	Linear regression
$y = 1.022x + 0.129$ mmol/L	$y = 1.020x - 0.205$ mmol/L
$\tau = 0.969$	$r = 0.999$

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

Urine

Sample size (n) = 68

Passing/Bablok ¹⁴	Linear regression
$y = 1.003x - 0.275$ mmol/L	$y = 1.013x - 0.628$ mmol/L
$\tau = 0.976$	$r = 0.999$

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 ¹⁴	Linear regression
$y = 1.017x + 0.00334$ mmol/L	$y = 1.017x - 0.0168$ mmol/L
$\tau = 0.978$	$r = 1.000$

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

Urine

Sample size (n) = 75

Passing/Bablok ¹⁴	Linear regression
$y = 1.006x + 0.0109$ mmol/L	$y = 1.005x + 0.0511$ mmol/L
$\tau = 0.993$	$r = 1.000$

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

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.

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Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
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Volume for reconstitution

GTIN	Global Trade Item Number
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Rx only

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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ETOH2

Ethanol Gen.2

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CE 0123



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