ETOH2

cobas®

Ethanol Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03183777 190	Ethanol Gen.2 (100 tests)	System-ID 07 6611 9	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:

ETOH2: ACN 703

SETH2: ACN 671 (STAT, reaction time: 5)

For **cobas c** 502 analyzer:

ETOH2: ACN 8703

SETH2: ACN 8671 (STAT, reaction time: 5)

Intended use

In vitro test for the quantitative determination of ethanol in human serum, plasma and urine on Roche/Hitachi **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 Redetzki[2], 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.

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

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

R2 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 R2 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.

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

Reagent handling

Ready for use

Storage and stability

ETOH2

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 K_2-EDTA

Stability:[5]	2 days at 25 °C
	2 weeks at 5 °C
	4 weeks at -15 °C

Plasma: NaF/Na_2-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 one 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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

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.

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Ethanol Gen.2

cobas®

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

cobas c 311 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 14-23 (STAT 5 / 14-23)	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Increase	
Units	mmol/L (g/L, mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	50 µL	–
R2	50 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	4 µL	–	–
Decreased	2 µL	–	–
Increased	4 µL	–	–

cobas c 501 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 21-33 (STAT 5 / 21-33)	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Increase	
Units	mmol/L (g/L, mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	50 µL	–
R2	50 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	4 µL	–	–
Decreased	2 µL	–	–
Increased	4 µL	–	–

cobas c 502 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 21-33 (STAT 5 / 21-33)	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Increase	
Units	mmol/L (g/L, mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	50 µL	–
R2	50 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	4 µL	–	–
Decreased	2 µL	–	–
Increased	8 µL	–	–

Calibration

Calibrators	S1: H_2O S2: Ammonia/Ethanol/CO2 Calibrator
Calibration mode	Linear
Calibration frequency	2-point calibration - after reagent lot change - after 6 weeks on board - 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 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.

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[8]

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

Conversion factors:	mmol/L x 0.04608 = g/L
	mmol/L x 4.608 = mg/dL
	g/L x 21.7 = mmol/L
	g/L x 100 = mg/dL

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

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 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

Serum, plasma and urine

2.20-108 mmol/L (0.101-4.98 g/L, 10.1-498 mg/dL)

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

Lower detection limit

2.20 mmol/L (0.101 g/L, 10.1 mg/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 the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Serum/plasma[8]

10.9-21.7 mmol/L (0.5-1 g/L, 50-100 mg/dL)	Flushing, slowing of reflexes, impaired visual acuity
> 21.7 mmol/L (> 1 g/L, > 100 mg/dL)	Depression of CNS
> 86.8 mmol/L (> 4 g/L, > 400 mg/dL)	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. 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:

Serum/plasma

Repeatability	*Mean*	*SD*	*CV*
	mmol/L (g/L, mg/dL)	*mmol/L (g/L, mg/dL)*	*%*
AEC Control N	10.9 (0.502, 50.2)	0.2 (0.009, 0.9)	1.6
AEC Control A	32.5 (1.50, 150)	0.3 (0.01, 1)	0.9
Human serum 1	19.7 (0.908, 90.8)	0.2 (0.009, 0.9)	1.2
Human serum 2	75.8 (3.49, 349)	0.8 (0.04, 4)	1.1
Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L (g/L, mg/dL)	*mmol/L (g/L, mg/dL)*	*%*
AEC Control N	11.1 (0.511, 51.1)	0.3 (0.01, 1)	2.4
AEC Control A	31.6 (1.46, 146)	0.4 (0.02, 2)	1.2
Human serum 3	26.9 (1.24, 124)	0.6 (0.03, 3)	2.0
Human serum 4	68.4 (3.15, 315)	0.8 (0.04, 4)	1.2

Urine

Repeatability	*Mean*	*SD*	*CV*
	mmol/L (g/L, mg/dL)	*mmol/L (g/L, mg/dL)*	*%*
AEC Control N	10.9 (0.502, 50.2)	0.2 (0.009, 0.9)	1.6
AEC Control A	32.5 (1.50, 150)	0.3 (0.01, 1)	0.9
Human urine 1	21.0 (0.968, 96.8)	0.3 (0.01, 1)	1.4
Human urine 2	76.5 (3.53, 353)	0.6 (0.03, 3)	0.8
Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L (g/L, mg/dL)	*mmol/L (g/L, mg/dL)*	*%*
AEC Control N	11.1 (0.511, 51.1)	0.3 (0.01, 1)	2.4
AEC Control A	31.6 (1.46, 146)	0.4 (0.02, 2)	1.2
Human urine 3	19.0 (0.876, 87.6)	0.4 (0.02, 2)	1.9
Human urine 4	34.5 (1.59, 159)	0.6 (0.03, 3)	1.8

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

Method comparison

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

Serum/plasma

Sample size (n) = 72

Passing/Bablok[14]	Linear regression
y = 1.023x + 0.090 mmol/L	y = 1.020x + 0.248 mmol/L
τ = 0.988	r = 1.000

The sample concentrations were between 2.67 and 94.1 mmol/L (0.123 and 4.34 g/L, 12.3 and 434 mg/dL).

Urine

Sample size (n) = 73

Passing/Bablok[14]	Linear regression
y = 1.008x + 0.288 mmol/L	y = 1.007x + 0.261 mmol/L
τ = 0.982	r = 1.000

The sample concentrations were between 2.85 and 97.1 mmol/L (0.131 and 4.47 g/L, 13.1 and 447 mg/dL).

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

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.

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

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

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Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336