ETOH2

Ethanol Gen.2

cobas®

Drug abuse testing

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	COBAS INTEGRA 400 plus

Materials required (but not provided):

20751995 190	Ammonia/Ethanol/CO_2 Calibrator (2 x 4 mL)	System-ID 07 5199 5	
20752401 190	Ammonia/Ethanol/CO_2 Control Normal (5 x 4 mL)	System-ID 07 5240 1	
20753009 190	Ammonia/Ethanol/CO_2 Control Abnormal (5 x 4 mL)	System-ID 07 5300 9	

English

System information

Test ETOH2, test ID 0-611 (serum, plasma)

Test ETOU2, test ID 0-511 (urine)

Intended use

In vitro test for the quantitative determination of ethanol in human serum, plasma, and urine on COBAS INTEGRA 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.

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

The NADH formed during the reaction, measured photometrically as a rate of change in absorbance, is directly proportional to the ethyl alcohol concentration. It is determined by measuring the increase in absorbance at 340 nm.

Reagents - working solutions

R1 Buffer; preservatives

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

R1 is in position B and SR 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

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
On-board in use at 10-15 °C	4 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-, Na-, NH_4^+-heparin and K_2-, K_3-EDTA plasma; NaF/Na_2EDTA and NaF/K-oxalate plasma

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.

Centrifuge samples containing precipitates before performing the assay.

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

Stability in serum and Li-, Na-, NH_4^+-heparin and K_2-, K_3-EDTA plasma:[5]	2 days at 25 °C 2 weeks at 5 °C 4 weeks at –15 °C
Stability in NaF/Na_2EDTA and NaF/K-oxalate plasma:[5]	2 weeks at 25 °C 3 months at 5 °C 6 months at –15 °C
Stability in urine:[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).

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.

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.

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.

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Drug abuse testing

Application for serum, plasma and urine

Test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/659 nm
Calc. first/last	44/54
Unit	mmol/L

Pipetting parameters

Serum, plasma, urine		Diluent (H_2O)
R1	50 µL	-
Sample	4 µL	16 µL
SR	50 µL	-
Total volume	120 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 **cobas c** pack and 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 standard materials.

Quality control

Quality control	Ammonia/Ethanol/CO_2 Control Normal and Abnormal
Control interval	8 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

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factor:[7] mmol/L × 4.61 = mg/dL

Limitations - interference

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.

Criterion: Recovery within ± 10 % of initial value.

Serum/plasma

Icterus:[8] 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:[8] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[8] No significant interference up to an L index of 1200. 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.[9,10]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[11]

LDH/lactic acid (using a dose-response curve with purified LDH fractions added to 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).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10] Exception: No significant interference from salicyluric acid up to a concentration of 600 mg/L.

Criterion: Recovery within ± 10 % of initial value at an ethanol concentration of 21.7 mmol/L (100 mg/dL)

Urines containing sugars and contaminated with microorganisms may yield a false positive result due to fermentation of sugar to alcohol.

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

2.17-108 mmol/L (10.0-498 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.

Lower limits of measurement

Lower detection limit of the test (serum, plasma, and urine):
2.17 mmol/L (10.0 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 a zero sample (zero sample + 3 SD, repeatability, n = 21).

Expected values

Serum/plasma[7]

10.9-21.7 mmol/L (50.2-100 mg/dL)	Flushing, slowing of reflexes, impaired visual acuity
> 21.7 mmol/L (> 100 mg/dL)	Depression of CNS
> 86.8 mmol/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.[12]

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 COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined on a COBAS INTEGRA 700 analyzer using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

Serum/plasma

	Repeatability		*Intermediate precision*	
Sample	*Mean mmol/L (mg/dL)*	*CV %*	*Mean mmol/L (mg/dL)*	*CV %*
Level 1	20.1 (93.0)	1.2	21.8 (100)	2.4
Level 2	42.0 (194)	1.1	42.8 (197)	3.9

Urine

	Repeatability		*Intermediate precision*	
Sample	*Mean mmol/L (mg/dL)*	*CV %*	*Mean mmol/L (mg/dL)*	*CV %*
Level 1	20.1 (93.0)	1.2	24.0 (111)	3.6
Level 2	31.9 (147)	1.7	30.7 (142)	3.3

The following results were obtained on a COBAS INTEGRA 400 analyzer with repeatability (n = 21) and intermediate precision (1 aliquot per run, 1 run per day, 10 days):

Serum/plasma

	Repeatability		*Intermediate precision*	
Sample	*Mean mmol/L (mg/dL)*	*CV %*	*Mean mmol/L (mg/dL)*	*CV %*
Level 1	24.2 (111.6)	0.8	23.8 (109.7)	1.0
Level 2	51.5 (237.4)	0.7	51.5 (237.4)	1.0

Method comparison

Serum/plasma

Ethanol values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Ethanol Gen.2 reagent (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and with those determined using the previous reagent (ETOH) on a COBAS INTEGRA 700 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 52
Passing/Bablok[13]	Linear regression
y = 0.958x + 0.242 mmol/L	y = 0.964x + 0.053 mmol/L
τ = 0.970	r = 0.999
SD (md 95) = 2.40	Sy.x = 1.06

The values were between 8.51 and 105 mmol/L (39.2 and 484 mg/dL).

COBAS INTEGRA 700 analyzer	Sample size (n) = 51
Passing/Bablok[13]	Linear regression
y = 0.957x - 0.474 mmol/L	y = 0.963x - 0.675 mmol/L
τ = 0.969	r = 0.999
SD (md 95) = 1.81	Sy.x = 0.818

The values were between 8.63 and 109 mmol/L (39.8 and 502 mg/dL).

Ethanol values for human serum and plasma samples obtained on a COBAS INTEGRA 400 analyzer using the COBAS INTEGRA Ethanol Gen.2 reagent (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and with those determined using the previous reagent (ETOH) on a COBAS INTEGRA 400 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 52
Passing/Bablok[13]	Linear regression
y = 0.982x + 0.485 mmol/L	y = 0.980x + 0.534 mmol/L
τ = 0.961	r = 0.998
SD (md 95) = 2.68	Sy.x = 1.43

The sample concentrations were between 8.30 and 106 mmol/L (38.3 and 489 mg/dL).

COBAS INTEGRA 400 analyzer	Sample size (n) = 52
Passing/Bablok[13]	Linear regression
y = 0.991x + 0.296 mmol/L	y = 0.997x + 0.079 mmol/L
τ = 0.989	r = 1.000
SD (md 95) = 0.977	Sy.x = 0.464

The sample concentrations were between 8.30 and 106 mmol/L (38.3 and 489 mg/dL).

Urine

Ethanol values for human urine samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Ethanol Gen.2 reagent (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and with those determined using the previous reagent (ETOH) on a COBAS INTEGRA 700 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 60
Passing/Bablok[13]	Linear regression
y = 0.964x - 0.217 mmol/L	y = 0.967x - 0.296 mmol/L
τ = 0.978	r = 0.999
SD (md 95) = 0.936	Sy.x = 0.779

The values were between 0.270 and 111 mmol/L (1.24 and 512 mg/dL).

COBAS INTEGRA 700 analyzer	Sample size (n) = 58
Passing/Bablok[13]	Linear regression
y = 0.997x - 0.235 mmol/L	y = 0.993x - 0.245 mmol/L
τ = 0.979	r = 0.999
SD (md 95) = 1.74	Sy.x = 0.699

The values were between 0.270 and 108 mmol/L (1.24 and 498 mg/dL).

Ethanol values for human urine samples obtained on a COBAS INTEGRA 800 analyzer (y) using the Ethanol Gen.2 reagent were compared with those determined using the corresponding reagent on a COBAS INTEGRA 400 Plus analyzer (x).

COBAS INTEGRA 400 Plus analyzer	Sample size (n) = 65
Passing/Bablok[13]	Linear regression
y = 0.979x - 2.20 mmol/L	y = 0.986x - 2.48 mmol/L
τ = 0.974	r = 0.997

The sample concentrations were between 0.24 and 105.1 mmol/L (1.11 and 485 mg/dL).

Analytical specificity

COBAS INTEGRA 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	8.5	4.8
n-Butanol	2.9	2.5
Isopropanol	0.5	0.3
Acetone	0.0	0.0
Ethylene glycol	0.0	0.0
Methanol	0.0	0.0
Acetaldehyde	0.0	0.0

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

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

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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