Ethanol Gen.2

Order information

COBAS INTEGRA Ethanol Gen.2	100 Tests	Cat. No. **03183777** 190 System-ID 07 6611 9
Roche Ammonia/Ethanol/CO_2 Calibrator	2 × 4 mL	Cat. No. **20751995** 190 System-ID 07 5199 5
Roche Ammonia/Ethanol/CO_2 Control Normal	5 × 4 mL	Cat. No. **20752401** 190 System-ID 07 5240 1
Roche Ammonia/Ethanol/CO_2 Control Abnormal	5 × 4 mL	Cat. No. **20753009** 190 System-ID 07 5300 9

● Indicates analyzer(s) on which **cobas c** pack can be used

COBAS INTEGRA 400/400 plus	COBAS INTEGRA 800
●	●

System information
COBAS INTEGRA Ethanol Gen.2 (ETOH2)
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.

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

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 (liquid)
R2 = SR NAD/ADH (liquid)

Components	Concentrations R1	R2 = SR	Test	
NAD (yeast)		≥ 3	≥ 1.25	mmol/L
ADH (EC 1.1.1.1, yeast, 25 °C)		≥ 37	≥ 15.42	U/mL

Both reagents contain nonreactive stabilizers and preservatives.

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

Reagent handling
Ready for use.

Storage and stability

Shelf life at 2 to 8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400/400 plus systems	
On-board in use at 10 to 15 °C	4 weeks
COBAS INTEGRA 800 systems	
On-board in use at 8 °C	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, and urine
Plasma: Li-, Na-, NH_4^+-heparin and K_2-, K_3-EDTA, NaF/Na_2EDTA and NaF/K-Oxalate.

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.

Storage
Samples must be tightly closed. Sodium fluoride, 1 % (w/v), is the most satisfactory preservative. With sodium fluoride preservative, the specimen is reliable for 2 weeks at 25 °C, 3 months at 5 °C and 6 months at -15 °C.[5]
Centrifuge samples containing precipitates before performing the assay.

Materials provided
See "Reagents - working solutions" section for reagents.

Assay

For optimal performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

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

When using Ammonia/Ethanol/CO_2 Calibrator: Do not leave calibrator cups open for longer than 30 minutes at 15-25 °C.

When using Ammonia/Ethanol/CO_2 Controls: Do not leave control cups open for longer than 1 hour at 15-25 °C.

Application for serum, plasma and urine

COBAS INTEGRA 400/400 plus 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	

COBAS INTEGRA 800 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	62/79
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	Roche Ammonia/Ethanol /CO_2 Calibrator Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	**COBAS INTEGRA 400/400 plus systems**: Each **cobas c** pack and as required following quality control procedures **COBAS INTEGRA 800 system**: Each **cobas c** pack, every 6 weeks, and as required following quality control procedures

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

Quality control

Quality control	Roche 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. Other suitable control material can be used in addition.
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 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 400/400 plus/800 analyzers).

Conversion factor[5]: 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.
Urines containing sugars and contaminated with microorganisms may yield a false positive result due to fermentation of sugar to alcohol.

Criterion: Recovery within ± 10 % of initial value.

Serum, plasma

Icterus[6]	No significant interference with conjugated and unconjugated bilirubin
Hemolysis[6]	No significant interference
Lipemia[6]	No significant interference
Drugs	No interference was found at therapeutic concentrations using common drug panels.[7,8]
Other	In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Urine

Drugs	No interference was found at therapeutic concentrations using common drug panels.[8] *Exception:* Hypochlorite causes interference.

Note

Other similar alcohol reagents may give falsely elevated results with samples containing extremely high levels of both LD and lactic acid, especially post mortem samples.[9]
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 Method Manual, Introduction, Extra Wash Cycles for further instructions.
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 by 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 three standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 21).

Expected values[5]

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

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[a] (n = 21) and intermediate precision[b] (1 aliquot per run, 1 run per day, 21 days).
The following results were obtained:

Serum/plasma

	Repeatability[a]		Intermediate precision[b]	
Sample	Mean	CV	Mean	CV
	mmol/L (mg/dL)	%	mmol/L (mg/dL)	%
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[a]		Intermediate precision[b]	
Sample	Mean	CV	Mean	CV
	mmol/L (mg/dL)	%	mmol/L (mg/dL)	%
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

a) repeatability = within-run precision
b) intermediate precision = total precision / between-run precision / between-day precision

Method comparison

Serum/plasma

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

Roche/Hitachi 917 analyzer	Sample size (n) = 52
Passing/Bablok[10]	Linear regression
$y = 0.958x + 0.242$ mmol/L	$y = 0.964x + 0.053$ mmol/L
$\tau = 0.970$	r = 0.999
SD (md 95) = 2.40	Sy.x = 1.06

Values ranged from 8.51 to 105 mmol/L (39.2 to 484 mg/dL).

COBAS INTEGRA 700 analyzer	Sample size (n) = 51
Passing/Bablok[10]	Linear regression
$y = 0.957x - 0.474$ mmol/L	$y = 0.963x - 0.675$ mmol/L
$\tau = 0.969$	r = 0.999
SD (md 95) = 1.81	Sy.x = 0.818

Values ranged from 8.63 to 109 mmol/L (39.8 to 502 mg/dL).

Urine

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

Roche/Hitachi 917 analyzer	Sample size (n) = 60
Passing/Bablok[10]	Linear regression
$y = 0.964x - 0.217$ mmol/L	$y = 0.967x - 0.296$ mmol/L
$\tau = 0.978$	r = 0.999
SD (md 95) = 0.936	Sy.x = 0.779

Values ranged from 0.270 to 111 mmol/L (1.24 to 510 mg/dL).

COBAS INTEGRA 700 analyzer	Sample size (n) = 58
Passing/Bablok[10]	Linear regression
$y = 0.997x - 0.235$ mmol/L	$y = 0.993x - 0.245$ mmol/L
$\tau = 0.979$	r = 0.999
SD (md 95) = 1.74	Sy.x = 0.699

Values ranged from 0.270 to 108 mmol/L (1.24 to 498 mg/dL).

Analytical specificity

COBAS INTEGRA Ethanol Gen.2 reagent is specific for ethyl alcohol. The following cross reactants were measured at 2000 mg/dL.

Compound	% Cross-reactivity (urine)
Acetaldehyde	-1.6
Acetone	0.0
N-butanol	0.1
Ethylene glycol	0.1
Isopropanol	0.3
Methanol	0.0
N-propanol	6.0

$$\text{Cross-reactivity (\%)} = \frac{100 \times (\text{analytical result - analyte concentration})}{\text{concentration of interferent}}$$

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