ETHANOL

Diagnostic reagent for determination of Ethanol concentration.
Liquid. Dual reagents. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
HN395	200 mL	LTH1	200 mL	M3110	250 mL	ZA21	200 mL
HN396	100 mL	LTH2	100 mL	M4110	100 mL	ZA23	100 mL
LB100	150 mL	LTH3	120 mL	S2473	100 mL	8A2163	250 mL
LM248	150 mL	ME110	100 mL	TTH1	200 mL	DDH1	160 mL
LM259	150 mL	ME111	100 mL	TTH2	100 mL	DDH2	128 mL
LM278	160 mL	ME210	96 mL				

Changes made in the instructions for use are marked as grey.

INTENDED USE

This test is used for the quantitative determination of ethanol in serum and plasma in biochemistry autoanalyzers.

GENERAL INFORMATION

Ethanol is the most widely used and often abused chemical substance. It is one of the most common tests performed in a toxicology laboratory. Ethanol is considered a central nervous system (CNS) depressant whose effects vary depending on its concentration in the blood. However, it is also greatly influenced by interindividual differences in tolerance. Symptoms range from euphoria and decreased inhibition to increased disorientation and incoordination, followed by coma and death. Many countries set a blood alcohol concentration of 0.8 g/L (0.08 g/100 mL or 0.08% w/v, 17.4 mmol/L) or less (0.5 g/L) as the legal limit for driving a motor vehicle.[1] Due to many factors, not all individuals experience the same degree of CNS dysfunction at similar blood alcohol concentrations. Furthermore, the CNS effects of ethanol are more distinctive when the concentration of ethanol in the blood increases (absorption phase) than when it decreases (elimination phase), partly due to the phenomenon of acute tolerance.[2] In addition, excessive alcohol use leads to the development of a chronic tolerance. Ethanol, when consumed in combination with other CNS depressant drugs, exerts a potentiating or synergistic depressant effect. This can occur at relatively low alcohol concentrations, and a large number of deaths are due to the combination of ethanol and drug ingestion.[3]

The pharmacological mechanisms of the CNS depressant effects of ethanol are complex and not fully understood, but likely involve both enhancement of major inhibitory neurons and disruption of excitatory neurons. The major CNS inhibitory neuron system is mediated by the neurotransmitter Ɣ-aminobutyric acid (GABA). When GABA binds to the postsynaptic receptor subtype GABAA, this oligomeric ion-gated complex "opens" to allow inward influx of Cl, leading to membrane hyperpolarization and subsequent reduction of the electrical response.[1] Neuronal nicotinic acetylcholine receptors may also be prominent molecular targets of alcohol.[4]

Both enhancement and inhibition of nicotinic acetylcholine receptor function have been reported, depending on receptor subunit concentration and ethanol concentrations tested.[1] Ethanol also inhibits the function of Nmethyl-d-aspartate (NMDA)- and kainate-receptor subtypes; however, -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors are largely resistant to ethanol.[5]

The aforementioned chronic tolerance to ethanol is thought to be mediated by ethanol-induced increased responsiveness and upregulation in the synthesis of NMDA receptors, together with their downregulation and desensitization through simultaneous phosphorylation of $GABA_A$ and glutamate receptors.[6-8] Largely due to these adaptive changes, abrupt cessation of chronic, heavy ethanol use leads to physical withdrawal syndrome, which is the opposite of the hallmarks of intoxication. Symptoms include CNS stimulation, anxiety, irritability, nervousness, insomnia, muscle tremors and cramps, hallucinations and increased fever, blood pressure, heart rate and seizures.[5] This syndrome can be fatal if the patient is not properly monitored.[1]

Ethanol is mainly metabolized to acetaldehyde by liver alcohol dehydrogenase (ADH) and then oxidized to acetic acid by aldehyde dehydrogenase. The rate of elimination of ethanol from the blood is close to a zero-order process. This rate varies between individuals, averaging 0.15 g/L/h in men and 0.18 g/L/h in women.[9,10] At both low (0.2 g/L)[11] and high (3 g/L) ethanol concentrations, elimination more closely resembles first-order kinetics and is accelerated at higher concentrations.[12] The elimination rate is also influenced by drinking habits (e.g. increased elimination rates in alcoholics caused by enzyme induction).[13]

Ethanol is teratogenic and alcohol consumption during pregnancy can result in a baby with fetal alcohol spectrum disorders. These effects can include physical, mental, behavioral and/or learning disorders with possible lifelong consequences and are 100% preventable when a woman abstains completely from alcohol during pregnancy.[1,14]

TEST PRINCIPLE

Enzymatic – colorimetric method

Enzymatic assay is the preferred method of many laboratories to measure ethanol in serum/plasma. In this method, ADH catalyzes the oxidation of ethanol to acetaldehyde and NAD is reduced to NADH. The formation of NADH by this reaction can be measured at 340 nm and is proportional to the amount of ethanol in the sample.

$$Ethanol + NAD \xrightarrow{ADH} Acetaldehyde + NADH$$

REAGENT COMPONENTS

Reagent 1:

Buffer	≤ 260 mol/L
Stabilizers	
Preservatives	

Reagent 2:

Buffer	≤ 10 mmol/L
NAD	> 2 mmol/L
Alcohol dehydrogenase (ADH)	> 40 Ku/L
Stabilizers	
Preservatives.	

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[15]

SAMPLE REQUIREMENTS

Serum and plasma are collected by standard procedure. Li heparin and K_2 EDTA sample collection tubes should be preferred for plasma. Multiple samples freezing and thawing should be avoided.

Ethanol activity stability in serum and plasma[31]:

2 days at +20/+25 ºC,
2 weeks at +2/+8ºC,
4 weeks at -20ºC.

Note 1: Serum and plasma are suitable samples for the determination of ethanol. Alcohol is distributed in the aqueous portions of blood; since serum has a higher water content than whole blood, higher alcohol concentrations are obtained from serum compared to whole blood. Experimentally, the serum/whole blood ethanol ratio is 1.18 (1.10 to 1.35)[16] and varies slightly by hematocrit.[17] Therefore, laboratories performing alcohol determinations should clearly specify sample selection.

Note 2: Ethanol may evaporate. Samples should be tightly capped to prevent evaporation.

Note 3: For longer storage requirements (witness samples in forensic cases, study blood, etc.) or for non-sterile post-mortem samples, sodium fluoride should be used as a preservative to minimize changes in ethanol concentration.[1]

Unit Conversion:
mg/dL × 0.217 = mmol/L
mmol/L x 0.04608 = g/L
mmol/L x 4.608 = mg/dL
mmol/L x 0.0374 = ‰

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Ethanol Calibrator or Ammonia/Ethanol/Bicarbonate Calibrator Set.

Ethanol Calibrator (Liquid)
Ref.No: ELCL4

Ammonia/Ethanol/Bicarbonate Calibrator Set (Liquid)
Ref.No: ZA92

Calibration stability is 13 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Ethanol Control Level I (Liquid)
Ref.No: ELCN1

Ethanol Control Level II (Liquid)
Ref.No: ELCN2

Ammonia/Ethanol/Bicarbonate Control Set
Ref.No: ELCN3

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Ethanol is only found in serum or plasma after external intake.

30 - 120 mg/dL:	Slowed reflexes; lack of attention, assessment and control
120 - 250 mg/dL:	Decreased visual sensitivity and increased reaction time
250 - 350 mg/dL:	Muscular incoordination, decreased response to stimuli

> 350 mg/dL: Impaired circulation and respiration, risk of death

The legal definition of Alcohol Poisoning varies according to local laws. Each laboratory should determine an acceptable reporting format and define procedures for reporting abnormal results. Clinical judgement and a professional approach should be applied in the evaluation of ethanol test results.
Reference interval data have been verified by using CLSI EP28-A3c protocol.[19]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[20]

The determined analytic measuring interval for Ethanol is 20 -500 mg/dL.

Detection Capability

Limit of Detection (LoD): 10 mg/dL

Limit of Quantitation (LoQ): 20 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[21]

Linearity

This method shows measurement linearity in the activities up to 500 mg/dL. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[22]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[23]
Repeatability (Within Run) and Repeatability (Day to Day) SD and CV% values of Ethanol have been given in the table 1 and 2 respectively.

Table 1. Ethanol Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD*	CV%	n
0.51 g/L	0.01	1.96	80
0.98 g/L	0.02	2.04	80
1.99 g/L	0.01	0.50	80

***SD: Standard Deviation**

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[24]

Table 2. Ethanol Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
0.51 g/L	0.02	3.92	80
1.01 g/L	0.02	1.98	80
1.99 g/L	0.03	1.51	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[24]

Interference

Endogenous interferant and analyte concentrations that have been used in the Ethanol scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[25,26]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Ethanol interference scanning test is appropriate, is determined as ±10%.[27]

In Ethanol test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Ascorbic Acid	: < 30 mg/dL
Bilirubin	: < 60 mg/dL
Lipemia / Triglycerides	: < 2000 mg/dL
Hemoglobin	: < 2000 mg/dL
Urea	: < 2000 mg/dL
LDH	: < 2000 mg/dL

Note 1: Under normal assay conditions, isopropanol, acetone, methanol and ethylene glycol produce an acceptable (less than 1%) interference with the ADH enzyme present in reagent 2, which catalyzes its reaction in the assay.[1]

Note 2: When sampling for alcohol measurement, non-alcohol based disinfectants (e.g. aqueous benzalkonium, chlorhexidine) should be used at the blood collection site. If not available, the sampling site should be allowed to dry for 30-60 seconds to minimize the risk of interference.[1,28]
Note 3: False high results for ethanol have been reported in the presence of high lactate dehydrogenase (LDH) concentration along with high lactate concentration.[29,30] The basis of the interaction is due to an increase in the concentration of lactate in the blood with increasing LDH concentration. Increased lactate concentration may result from both clinical pathologies and trauma, especially tissue hypoperfusion.[1]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[26]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032	: Releases a very toxic gas if contacts with acid.
H317	: May cause allergic skin reaction.

Precaution

P280	: Use protective gloves / clothes / glasses / mask.
P264	: Wash your hands properly after using.
P272	: Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	: Wash with plenty of water and soap if it contacts with skin.
P333+P313	: Seek medical help if it irritates your skin or develops rash.
P362+P364	: Remove contaminated clothes and wash properly before using.

Disposal

P501	: Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests