ALCOHOL (Ethanol)

Enzymatic UV method - Endpoint

R1: 4 x 25 ml + R2: 1 x 25 ml CL28-125S

INTENDED USE

Kit for enzymatic determination of alcohol in serum and plasma.

CLINICAL MEANING

Ethanol is a depressor of the Central Nervous System: its effects on CNS are proportional to the concentration in plasma. In lower concentrations, alcohol can give euphoria and excitement. If the quantity is increased, the effects can be confusion, numbness and, in the worst cases, coma and death caused by respiratory palsy. The action at level of GABAergic receptors is similar to that of benzodiazepine and barbiturates.

PRINCIPLE

The enzymatic method described uses ethanol measuring according to the following reaction:

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

NADH absorbance, spectrophotometrical defined at 340 nm, is proportional to alcohol concentration in the sample.

SAMPLE

Serum and plasma. Use heparin or EDTA as anticoagulants.
STABILITY: 2 weeks at 2-8°C, 6 months at 2-8°C or -20°C.
Store samples tightly closed to avoid evaporation.
Do not use alcohol or volatile disinfectants during the measurement of ethanol.

REAGENTS

Only for in Vitro diagnostics. Liquid reagents ready to use.

Package content	**CL28-125S**
REAGENT 1 Buffer ph 9.0, 240 mM Stabilizers and preservatives	4 x 25 ml
REAGENT 2 Buffer ph 6.6 NAD, ADH, stabilizers and preservatives	1 x 25 ml
STANDARD Ethanol standard (concentration on the label) Recap the bottle immediately after use to prevent changes in concentration due to evaporation.	1 x 4 ml

WARNING: Recap the bottles immediately after use to prevent changes in concentration due to evaporation.
STABILITY: stored at 2-8°C, reagents are stable up to the expiration date indicated on the label if contamination is avoided. The standard is stable up to the expiration date indicated on the label even if stored at 15-25°C.

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

MANUAL ASSAY PROCEDURE

Wavelength:	340 nm
Optical path:	1 cm
Reading:	against air or distilled water
Temperature:	37°C
Reaction time:	10 minutes
Linearity:	up to 3,5 g/l
Reagent/Sample:	250/1

Let reagents and samples reach room temperature before use.

Pipette in cuvette or test tubes labeled as it follows:

	B/R	Std	Sample
Reagent 1	2,0 ml	2,.0 ml	2,0 ml
Demineralised water	10 µl	--	--
Standard	--	10 µl	--
Sample	--	--	10 µl

Mix with care. Incubate for 5 minutes at 37°C.
Read absorbance A1, then add:

Reagent 2	0,5 ml	0,5 ml	0,5 ml

Stir with care and incubate at 37°C. After exactly 10 minutes read absorbance A2.

CALCULATION

Calculate concentration in the sample using the following formulas:

Alcohol (ethanol) [g/L]= ($\Delta As - \Delta A_{B/R}$ / $\Delta Ast - \Delta A_{B/R}$) x Std concentration [g/L]

Alcohol (ethanol) [mmol/L]= ($\Delta As - \Delta A_{B/R}$ / $\Delta Ast - \Delta A_{B/R}$) x Std conc. [mmol/L]

REFERENCE VALUES

Ethanol is present in serum and blood only after ingestion.

g/L	(mmol/L)	Symptoms
0,5-1	(10.9-21,7)	Flushing, slowed reflexes, impaired visual activity
> 1	(>21,7)	Central nervous system depression (CNS)
> 4	(>86,8)	fatalities reported (i.e. respiratory failure)

QUALITY CONTROL – CALIBRATION

A quality control program is recommended for all clinical laboratories. Control serums in normal and high value ranges for each assay are recommended. The values obtained should fall within the manufacturer's accepted ranges for this method. Calibration with Standard included in the kit is recommended for every analysis.

PERFORMANCE CHARACTERISTICS

Sensitivity: The sensitivity of the method is about 0,10 g/l.

Linearity: up to 3,5 g/l (76 mmol/L).

For higher values, dilute samples with saline solution and multiply the result by the dilution factor.

Precision

Within run (n=10)	Average [g/L]	SD	CV %
Sample 1	0,435	0,009	2,12
Sample 2	1,96	0,043	2,18

Between run (n=20)	Average [g/L]	SD	CV %
Sample 1	0,440	1,99	2,27
Sample 2	0,008	0,05	2,5

Interferences:

No interference was observed by ascorbic acid up to 30 mg/dl, bilirubin up to 60 mg/dl, lipemia up to 2000 mg/dl triglycerides, hemoglobin up to 1000 mg/dl, creatinine up to 250 mg/dl, glucose up to 2000 mg/dl, urea p to 2000 mg/dl and LDH up to 2000 U/l.
Some alcohols interfere with the determination but react more slowly than ethanol. To avoid any interference, respect incubation time stated in the procedure.

Correlation against a reference method:

Correlation of FAR (Y) method with another kit available on the market (X) gave the following result:

Y = 1.041 X – 0.0256 r = 0.9987

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

Contact with the skin and ingestion should be avoided. Use the normal precautions expected with correct behavior in laboratory.

NOTE

Not everyone that has the same alcohol level in the blood presents an equal degree of CNS dysfunction. Every nation has set its limits on alcohol concentration in the blood of people driving vehicles.

REFERENCES

1. Gadsen R.H. et al. Ethanol in Biological Fluids by Enzymic Analysis. In : Selected Methods of Emergency Toxicology. C.S. Frings, W.R. Faulkner, Eds. Vol 11. Selected Methods of Clinical Chemistry, Washington DC, AACC Press, 1986, p. 63-65.

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KEY SYMBOLS