ETHANOL

Enzymatic UV

Summary:

The determination of ethanol belongs to the most frequent analyses in the forensic and toxilogical laboratory. It serves for the diagnosis of intoxications and poisonings particularly for emergency room patients.

Method:

Enzymatic UV test with alcohol dehydrogenase (ADH)

Principle:

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

In the presence of NAD Ethanol is converted by the alcohol dehydrogenase The measured absorbance of the produced NADH is proportional to the ethanol concentration in the sample

Reagents:

Components and Concentrations
N.B. Concentrations are those in the final test mixture
R1: Buffer pH 9.0 240 mmol/l
Stabilizers and preservatives
R2: Buffer Ph 6.6 8 mmol/l
NAD ≥2 mmol/l
Alcohol dehydrogenase ≥40 kU/l

Storage Instructıons And Reagent Stabilitiy

The reagents ara stable up to the end of the indicated month of expiry, if stored at 2-8 ºC and Contaminations is avolded. Do not freeze the reagents!The standarts are stable up to the end of the indicated month of expiry, if stored at 15 – 25 ºC.

Warnings And Precautions

1.The reagents contain sodium azide (0.95 g/l) as preservative.Do not swallow! Avoid contact with skin and mucous membranes.
2. Take the necessary precautions for the use of laboratory reagents

Waste Management

Please refer to local legal requirements.

Reagent Preperatıon

The reagents and the standarts are ready to use.

Materials Required But Not Provided

NaCl solution (0,9 %).
General laboratory equipment

Assay Procedure

Application sheets for automated systems are availlable on request.
Wavelenght 376 nm(360-380)
Optical path 1 cm
Temperature 37 ºC
Measurement Against reagent blank
The observance of exact measuring times and absolute equal treatment of all samples,standarts and controls must be respected

Reagent blank	**Sample /Standart**
Sample / Standart	– 10 µl
Dist. Water	10 µl –
Reagent I	1000 µl 1000 µl

Mix and incubate 5 min. at 37 ºC Read absorbance A1 then add

Reagent II	1000 µl	1000 µl

Mix and incubate 5 min. at 37 ºC Read absorbance A2 immediately.

A= (A2 – A1) Sample /Standart

Specimen

Serum and plasma (heparin and EDTA)

The stability in serum and plasma is 2 weeks at 20-25 ºC, 6 months at 4-8 ºC. Samples must be stored tightly closed.Don't use alcohol or volatile disinfectants during ethanol measurement.Discard contaminated specimens!

Calculations

One point or multi-point calibration.
One point calibration:
With standart 1.0 g/l ;

$$\text{A Sample} \times \text{C Standard (g/l)}$$
$$\text{A Std}$$

Multi-Point Calibration

The ethanol concentration of unkwon samples is derived from a calibration curve using a linear Algorithm. The calibration curve is obtained with four standats at different levels (see order information)and NaCl solution (0,9 %) for determination of the zero value.

Conversion Factor

Ethanol (g/l) *21.7 = Ethanol (mmol/l)
Ethanol (g/l)* 0.8 = Ethanol %

Performance Characteristics

Intra assay n=20	Mean g/l	SD g/l	CV %
Sample 1	0.51	0.01	1.67
Sample 2	0.98	0.02	1.95
Sample 3	1.99	0.01	0.66
Inter assay n=20	**Mean g/l**	**SD g/l**	**CV %**
Sample 1	0.51	0.02	3.36
Sample 2	1.01	0.02	2.05
Sample 3	1.99	0.03	1.66

Measuring Range

The test has been developed to determine ethanol concentrations up to 3.5 g/l.When values exceed this range samples should be diluted 1+1 with NaCl solution (9 g/l) and the result multiplied by

Specifity intereferences

No interference was observed by ascorbic acid up to 30 mg/dl,bilirubin up to 60 mg/dl , lipemla 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 up to 2000 mg/dl and LDH up to 2000 U/l.

Sensivity / Limit of Detection

The lower limit of dedection is 0.1 g/l.
Precision (at 37 ºC)

Method Comparison

A comparison between BIOANALYTIC Ethanol (y) and acommercially available assay (x) using
30 samples gave following results : y= 1.0 * + 0.1 g/l ; r = 0.999.

Referance Range

Result < 10 mg/dL Negative result
10 – 30 mg/dL 2.17 – 6.5 mmol/L Without obvious signs
30 – 120 mg/dL 6.5 – 26.0 mmol/L Slowed reflexes, diminution of attention, judgment and control
120 – 250 mg/dL 26.0 – 54.3 mmol/L Reduced visual acuity and increased reaction time
250 – 350 mg/dL 54.3 – 76.0 mmol/L Muscular incoordination, decreased response to stimuli
> 3.5 g/L > 76.0 mmol/L Impairment of circulation and respiration, possible death

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Quality Control:

Control Serum: BIOANALYTIC ETH CON.

SET 5 x 1 ml #B10827

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:

Standardization: This Ethanol method was calibrated against an international standard defined for Ethanol.

S1:BIOANALYTIC ETH CAL. SET 5 x 1 ml #B11914

Calibration frequency:

Calibration is recommended
after lot change
as required following quality control procedures

Bibliography

1. Borque L, Rus A, Dubois H. Automated determination of streptolysin O antibodies by turbidimetric latex immunoassay method. J Clin Immunoassay 1992; 15: 182-6.
2.Thomas L. Clinical Laboratory Diagnostics I ed Frankfurt: TH-books Verlagsgesellschaft,1998.
p.1168 – 1170 .
3. Immunology and Serology in Laboratory Medicine, 2nd edition. Turgeon ML. Mosby, 1996.

Order information (Cat No.) :

CR535	B24146	B28145	B31146	B34145	B36146
B21145	B25145	B28146	B32145	B34146	B37145
B21146	B25146	B30145	B32146	B35145	B37146
B22145	B27145	B30146	B33145	B35146	B80145
B24145	B27146	B31145	B33146	B36145	

Manufacturer

Diaclinica Diagnostik Kimya.San.Tic.Ltd.Şti
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SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions

ISO 9001:2015
ISO 13485:2016