ETHANOL FL

EH F060 CH 6 x 10 ml

INTENDED USE

Reagent for quantitative in vitro determination of ethanol in biological fluids.

SUMMARY OF TEST

Ethanol (ethyl alcohol) is a widely used and often abused chemical substance. The main pharmacological action of ethanol is the depression of CNS. When consumed with other CNS depressant drugs, ethanol has a potentiating or synergistic depressant effect. The mechanisms of depressant action of ethanol are complex and not fully understood, but likely deal with enhancement of main inhibitory neurons and impairment of excitatory neurons.

PRINCIPLE OF THE METHOD

Ethanol is oxidized by enzyme Alcohol Dehydrogenase (ADH) in the presence of NAD. Absorbance increase per unit time, due to formation of NADH, is proportional to the concentration of sample ethanol and can be monitored at 340 nm.

KIT COMPONENTS

For in vitro diagnostic use only.
The components of the kit are stable until expiration date on the label at 2-8°C.
Keep away from direct light sources.

ETOH R1 F060: 6 x 8 ml (liquid) blue cap

ETOH R2 F060: 1 x 12 ml (liquid) red cap

Test composition: Good's buffer 1 M, NAD ≥ 1 mM, ADH ≥ 5 kU/l, stabilizers and preservatives.

Standard: ethanol solution 200 mg/dl - 10 ml

Store all components at 2-8°C.
Ethanol is volatile! Immediately after use, close carefully the Standard dropper.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Sample as starter procedure:
Mix 1 part of reagent R2 with 4 parts of reagent R1.

Stability of working reagent: preferably within 90 days at 2-8°C, away from light sources.

Reagent as starter procedure:
Use separate reagents ready to use.
Stability: up to expiration date on labels at 2-8°C.
Stability since first opening of vials: use preferably within 60 days at 2-8°C.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.
Do not use alcohol or other volatile disinfectants during blood collection or storage.
Keep the sample tubes well capped, to avoid ethanol evaporation.

SPECIMEN

Serum, plasma. Urine.
Serum and plasma specimens can be stored 2 weeks at 2-25°C, up to 6 months at -20°C.

TEST PROCEDURE (sample as starter)

Wavelength:	340 nm
Lightpath:	1 cm
Temperatur:	37°C

dispense:	standard	sample
working reagent	1 ml	1 ml
standard	40 µl	-
sample	-	40 µl

Mix, after 90 seconds read the absorbance against water, by incubating at 37°C. Perform other two readings at 60 seconds intervals. Calculate the ΔA/min.

TEST PROCEDURE (reagent as starter)

Wavelenght:	340 nm
Lightpath:	1 cm
Temperature:	37°C

dispense:	standard	sample
reagent R1	1 ml	1 ml
standard	50 µl	-
sample	-	50 µl

Mix, incubate at 37°C for 5 minutes.

dispense:	standard	sample
reagent R2	250 µl	250 µl

Mix, after 90 seconds read the absorbance against water, by incubating at 37°C. Perform other two readings at 60 seconds intervals. Calculate the ΔA/min.

RESULTS CALCULATION

serum/plasma/urine:

$$\text{ethanol mg/dl} = \frac{\Delta A/min_{(sample)}}{\Delta A/min_{(standard)}} \times \text{standard value}$$

EXPECTED VALUES

30 - 120 mg/dl	euphoria, diminution of attention and control
120 - 250 mg/dl	excitement, reduced perception, increased reaction times
250 - 400 mg/dl	confusion, disturbed vision, muscular incoordination
> 400 mg/dl	unconsciousness, possibile death

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following control kit is available:
ETHANOL CONTROL SET

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity
The method is linear up to 600 mg/dl.
If the value is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)
The limit of detection is 2.7 mg/dl.

Interferences
In serum, no interference was observed by the presence of:

hemoglobin	≤ 500 mg/dl
bilirubin	≤ 33 mg/dl
lipids	≤ 1600 mg/dl
ascorbic acid	≤ 53 mg/dl

In urine, no interference was observed by the presence of:

hemoglobin	≤ 500 mg/dl
glucose	≤ 1780 mg/dl
urea	≤ 4600 mg/dl
creatinine	≤ 630 mg/dl

Samples with levels of Lactate 19 mM and LDH 7 KU/l do not produce interference. Higher levels of these components in the sample cause elevated results in ethanol assay.

Specificity
The reagent is specific for ethanol. Cross-reactions were observed at concentration 2000 mg/dl of the following compounds:

compound	cross-reaction % serum	urine
n-propanol	12.6	12.1
i-propanol	0.4	0.4
n-butanol	2.0	3.1
acetone	0.0	0.0
methanol	0.0	0.0

Precision

intra-assay (n=10)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	40.4	0.50	1.24
sample 2	151.1	0.84	0.56

inter-assay (n=20)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	40.8	0.79	1.94
sample 2	151.5	2.32	1.53

Methods comparison
A comparison between Chema and a commercially available product gave the following results:

ethanol competitor = x
ethanol Chema = y

Serum (n=49)
y = 1.02 x - 3.95 mg/dl $r^2 = 0.999$

Urine (n=49)
y = 1.04 x - 5.40 mg/dl $r^2 = 0.999$

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

Tietz Textbook of Clinical Chemistry, Fourth Edition, Burtis-Ashwood-Bruns (2006), 1300-1304
Analytical Sciences 2007, 23, 439-443
Clin. Chem. 2008, 54(7), 1251-2

MANUFACTURER

Chema Diagnostica
Via Campania 2/4
60030 Monsano (AN) - ITALY - EU
phone +39 0731 605064
fax +39 0731 605672
e-mail: mail@chema.com
website: http://www.chema.com

SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use-by date
	caution
	consult instructions for use

CE