Manual
Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 11030 R 3 Powder for 5 ml
For 15 tests

Alcohol

Fully enzymatic, Endpoint method
Without deproteinization

Powder Reagent

Test principle

$C_2H_5OH + NAD^+ \xrightarrow{ADH} CH_3CHO + NADH + H^+$

Alcohol dehydrogenase ADH catalyzes the oxidation of ethanol to acetaldehyde with the concomitant reduction of NAD to NADH. The increase in absorbance at 340 nm is directly proportional to alcohol concentration in the sample.

Concentrations in the test

Reagent R		
Tris-Buffer, pH = 9.2	142	mmol/L
Aminoxy acetic acid	47	µmol/L
ADH	3.3	KU/L
NAD	3.3	mmol/L
Stabilizer		

Standard : The concentration as indicated on vial.

Stability and preparation of working reagent

Reagent R: *Powder.*
The Reagent is stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent:
Dissolve content of bottle R1 with 5 ml of deionized water.
Stability: 3 days at 2 - 8 °C if tightly stoppered.

Note: Don't use the reagent if moisture has penetrated into the vial and caking has occurred or if the working reagent has an initial absorbance greater than 0.500 versus a water blank at 340 nm.

Specimen collection and handling

1. Serum, plasma collected in heparin, EDTA, citrate, oxalate, or fluoride. For Whole Blood add fluoride 5 mg/dl as preservative.
2. The site of venipuncture should be disinfected only with aqueous disinfectants such as zephiran or merthiolate. Alcohol or other volatile disinfectants must not be used.
3. Samples may be stored at 2 - 8 °C for several days when well stoppered.

Standard

Ethanol STD. Cat. No. 16021

Quality control

Ammonia/Ethanol control level 1 Cat. No. 15191
Ammonia/Ethanol control level 2 Cat. No. 15201

Procedure

Wavelength	Hg 340 nm (334 - 365 nm)
Spectrophotometer	340 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against reagent blank
Reaction	end point

Assay

	Blank	Standard	Sample
Double dist. water	5 µl	--	--
Standard	--	5 µl	--
Sample	--	--	5 µl
Working Reagent	1000 µl	1000 µl	1000 µl
Mix well and cork test tubes. Incubate for 5 min. at 37°C, or 10 min. at 20 - 25 °C, and measure the absorbance (A) within 30 min.			

Calculation

$$\text{Conc.}_{\text{Ethanol}}\ (\text{mg/dl}) = \frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times \text{Conc.}_{\text{Standard}}\ (\text{mg/dl})$$

Linearity

Up to 300 mg/dl at 365 nm.
Up to 200 mg/dl at (334 - 340) nm.

Interferences

1. The major interference in this assay is from alcohol used in cleansing the skin prior to venipuncture.
2. A comparison of relative interference by various alcohols is listed below.

Alcohol reactive after 5 minutes at 30°C :

Ethanol	100 %
N – butanol	38.5 %
Isopropanol	6.6 %
Methanol	0.0 %
Ethylene glycol	1.4 %
Acetone	0.0 %

A complete listing of potential interferences can be found in Young et. al.

Precautions

1. The alcohol reagent is irritant. Handle with normal precautions. In case of contact, rinse with plenty of water.
2. Laboratory atmosphere has to be free from alcohol and aldehyde.

Reference range [3]

Adults	0 – 9 mg/dl	0 – 1.95 mmol/L

References

1. Gruner,O.(1967),Der Gerichtsmedizinische Alkoholnachweis,KarlHeymannsverlag.
2. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.
3. Mosby's diagnostic and laboratory test reference, Seventh edition, page420.

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