POINTE SCIENTIFIC, INC.
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Alcohol Reagent Set

Intended Use
For the quantitative determination of ethyl alcohol in serum or whole blood. For *in vitro* diagnostic use only.

Clinical Significance
Alcohol analysis is most frequently requested for emergency room patients to aid in the differential diagnosis of central nervous system depression, which can be due to alcohol intoxication. It is also requested for forensic medicine purposes.

Method History
An enzymatic method for ethyl alcohol, using alcohol dehydrogenase and NAD, was first described by Bonnishen et al.[1] Several modifications and improvements of this method have subsequently been reported. The present procedure is a modification of the original enzymatic procedure. Due to its speed and ease of use, enzymatic analysis of ethyl alcohol has become quite common in laboratories. But because of medicolegal issues, the analysis must also be highly accurate. Therefore, it is generally recommended that assays for ethyl alcohol be confirmed by another method such as gas chromatography.

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

Alcohol dehydrogenase (ADH) catalyzes the oxidation of ethanol to acetaldehyde with the concomitant reduction of NAD to NADH.[2] The change in absorbance at 340 nm is directly proportional to the alcohol concentration in the sample.

Reagents
Concentrations refer to reconstituted reagent: Alcohol dehydrogenase (yeast) 200,000 U/L, NAD 2.8 mM, Buffer, pH 9.2 ± 0.1, surfactant, preservative.

Reagent Preparation
Reconstitute reagent with the volume of distilled or deionized water specified on the vial label. Swirl gently to dissolve.

Reagent Storage
1. Store reagent at 2-8°C.
2. Reconstituted reagent is stable for seven (7) days if tightly stoppered and stored at 2-8°C.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. The alcohol reagent is an IRRITANT. Handle with normal precautions. In case of contact rinse with plenty of water. Refer to MSDS for any hazard or safety information.
3. The reagent should not be used if moisture has penetrated the vial and caking has occurred or the reagent has an initial absorbance greater than 0.500 versus a water blank at 340 nm.
4. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories", 2nd Ed., 1988, HHS Publication No. (CDC) 88-8395.

Specimen Collection and Storage
1. 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.
2. Whole blood may be collected using citrate, heparin or oxalate as the anticoagulant.[3] However, fluoride used at 5 mg/dl of blood is the best preservative. Samples may be stored at 2-8°C for several days and may not show appreciable loss of alcohol, when well stoppered.[4]
3. Serum samples may be assayed without deproteinization.
4. Specimen collection should be carried out in accordance with NCCLS M29-T2.[5] No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

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 @ 30°C)[6]	
Ethanol	100%
n-Butanol	38.5%
Isopropanol	6.6%
Methanol	0.0%
Ethylene Glycol	1.4%
Acetone	0.0%

3. A complete listing of potential references can be found in Young, et al.[7]

Materials Provided
Alcohol reagent.

Materials Required but not Provided
1. Accurate pipetting devices
2. Test tubes
3. Timer
4. Spectrophotometer able to read at 340 nm.
5. Water bath or Heating block (30°C or 37°C)
6. Sodium Chloride, 0.9% (w/v): To prepare, dissolve 0.9 gm of reagent grade sodium chloride in distilled or deionized water and bring to a volume of 100 ml.
7. Trichloroacetic Acid Solution (6.25% w/v) - if whole blood is to be measured.
8. Centrifuge - if whole blood is to be measured.

Procedure (Automated-General)
Wavelength	340nm
Temperature	37°C or 30°C
Assay Type	Endpoint
Sample/Reagent Ratio	1:201
Reaction Direction	Increasing
Incubation Time	5 minutes
Low Normal	0 mg/dl
High Normal	100 mg/dl

Application parameters for various automated instruments are available. Please contact the manufacturer's Technical Service Department for specific information.

Alcohol
Reagent Set

Procedure (Manual)

1. Reconstitute reagent according to instructions.
2. Pipette 1.0ml of reagent into the required test tubes.
3. Bring the reagent in the tubes to the selected temperature (30°C is recommended for manual assay) and add 5 uL of the standard, test and control samples to the respective tubes. To the blank tube, add 5 uL of 0.9 sodium chloride solution.
4. Mix gently. Incubate for 5 minutes.
5. At the end of the incubation period, read and record the absorbance of all tubes against the reagent blank at 340 nm. Use this absorbance reading in the calculations.

NOTE: To measure whole blood samples the specimen must be deproteinizes as follows:

1. Pipette 1.8ml Trichloroacetic Acid Solution into a centrifuge tube.
2. While swirling tube, slowly add 0.2ml sample.
3. Stopper the tube and mix vigorously. Allow to stand at room temperature for approximately five minutes.
4. Centrifuge (2000 rpm) for five minutes to obtain a clear supernatant.
5. This supernatant may be assayed as above. However, to account for the dilution of the specimen it is recommended to use a 1:10 dilution of standard for calibration of the assay.

Limitations

Alcohol concentrations greater than 400 mg/dl should be diluted 1:1 with normal saline. Multiply results by 2 to compensate for dilution.

Calibration

Use an NIST-traceable Ethanol Standard or serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Calculation

$$\text{Ethanol conc. (mg/dl)} = \frac{\text{Abs. Sample}}{\text{Abs. Std.}} \times \text{conc. of Std.}$$

Example Calculation: Abs of patient = 0.450, Abs. of standard = 0.500, Conc. of Standard = 100

$$\frac{0.450}{0.500} \times 100 = 90 \text{ mg/dl}$$

Quality Control

Controls are recommended to monitor the performance of the assay, providing a constant screening of the instrument, reagents and techniques. Commercially available control material with established values for alcohol may be used. The controls should be run at least with every working shift in which ethanol assays are performed. It is recommended that each laboratory establish their own frequency of control determination.

Expected Values

Patients abstaining from alcohol have non-detectable levels by most enzymatic or chromatographic methods.[8] Below are the blood alcohol ranges shown with the corresponding percentage of subjects designated as intoxicated. In a study of over 6000 subjects, indications are very few (about 4%) people are intoxicated at blood levels of 0.05%.[9]

Blood Alcohol Levels	Percentage Diagnosed as Intoxicated
0.1 – 0.15%	63%
0.15 – 0.2%	89%
0.2 – 0.25%	95%

Above based on the averaged results of several studies.[9] Serum alcohol concentrations are usually about 16% higher than blood samples from which they are derived.[10]

Performance

1. Assay Range: 0 - 400 mg/dl
2. Comparison: Testing performed on 100 specimens compared with a similar method for ethanol yielded a correlation coefficient of 0.999 with a regression equation of y = 1.01x – 1.01. Sample values ranged from 2 to 412. (Sy.x = 12.84)
3. Precision: Precision studies were performed following a modification of the guidelines contained in NCCLS document EP5-T2.[11]

Within Run			Run to Run		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
40.3	0.72	1.78%	40.6	1.36	3.34%
93.4	1.04	1.11%	89.0	3.27	3.67%
219.8	2.17	0.99%	224.7	3.71	1.65%

4. Sensitivity: The sensitivity for the Alcohol reagent was investigated by reading the change in absorbance at 340 nm for a saline sample, and serums with known concentrations. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the Alcohol reagent showed little or no reagent drift on a zero sample. Also, that an absorbance change of 0.006 was approximately equivalent to one mg/dl of ethanol.

References

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