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Alcohol Determination in the Clinical Laboratory

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Dubowski, Kurt M.: Alcohol determination in the clinical laboratory. Am J Clin Pathol 74: 747–750, 1980. Four methods for blood-alcohol analysis—gas chromatography, enzymatic oxidation with alcohol dehydrogenase, chemical oxidation with acid dichromate, and osmometry—are briefly reviewed from the point of view of the clinical laboratory. Advantages and limitations of these methods are discussed, and their key features are tabulated. The correlation of the results of blood-alcohol analyses with stages of alcoholic influence and their corresponding signs and symptoms is presented in tabular form. (Key words: Alcohol determination; Blood-alcohol analysis.)

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THE DETERMINATION OF ALCOHOL* in blood and other biologic specimens is a frequently requested service in clinical laboratories, and is often requested on an emergency basis in hospitals.

Breath is the preferred specimen whenever the patient is able to participate in the breath-alcohol analysis. The latter has the inherent advantages of simplicity, rapidity, and noninvasive sampling, and reflects the alcohol content of the arterial circulation, which is physiologically and clinically more significant than the venous blood-alcohol concentration, especially during active alcohol absorption. Current instrumentation includes devices employing gas chromatography, infrared absorptiometry, fuel cell catalysis, and solid-state gas sensing, as well as the older chemical oxidation devices.

All quantitative breath-alcohol instruments currently marketed employ end-expiratory breath. Features and performance of current breath-alcohol analyzers of potential applicability to clinical laboratory practice have been summarized.[6,12,13] Several of the newer devices are portable, battery-powered, and self-contained, and hence are especially practical for bedside or emergency-room use. When necessary or desirable, whole breath or the alcohol in a measured volume of breath can be readily stored on simple sorption columns for subsequent elution and analysis.[9] Measurement of alcohol in breath involves some special biologic and chemical considerations,[7] and requires a 15-min "deprivation" period preceding breath-sampling to ensure the absence of contamination from recently ingested alcohol in the oral cavity.

Blood is the most useful specimen when breath is not available, with plasma or serum being physiologically more appropriate specimens than whole blood. However, for certain forensic applications (*e.g.*, investigation of alleged traffic law violations) analysis of whole blood is required because statutory interpretations of the results of blood-alcohol analyses are universally based upon whole blood. Collection of plasma and whole blood requires the use of anticoagulants—heparin, disodium edetate, and sodium or potassium citrate or oxalate are suitable if the specimen is not to be stored. For specimens to be preserved, potassium oxalate (5 mg/ml of blood) and sodium fluoride (1.5 mg/ml of blood) are an appropriate anticoagulant and preservative combination for short-term storage at 5 C of initially sterile blood specimens.

The many methods for alcohol analysis in blood fall into about a dozen different categories, of which the four listed in Table 1 are the most obvious candidates for use in the clinical laboratory. In the author's view, osmolality measurement, while rapid and simple, is not a method of choice. Estimation of alcohol in serum requires the subtraction from the actual osmolality determination result (by freezing-point depression) of the assumed normal osmolality value.[14] Substantial discrepancies between the blood-alcohol concentrations (BACs) thus estimated and the actual alcohol concentrations are possible and have been documented.[2]

The other methods for blood-alcohol analysis listed in Table 1 are appropriate for use in clinical laboratories, and are listed in order of this author's preference. Alcohol analysis by gas chromatography can be performed with simple and relatively inexpensive instruments and is the current method of choice. Among its desirable features are rapidity, sensitivity, minimal requirements for sample manipulation or preparation, and inherently great selectivity for ethanol even with single column/single condition analyses. Essentially

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* The unmodified term "alcohol" in this article refers to ethanol.

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Table 1. Summary of Methods for Blood-alcohol Analysis

Method	Required Sample Treatment/ Separation	Specificity for Ethanol	Apparatus Requirements	Final Measurement	Limitations
Gas chromatography	Dilution or headspace equilibration	Selective for ethanol; multiple columns/conditions greatly increase selectivity	Gas chromatograph, water bath (for headspace), strip chart recorder or integrator	Electrical voltage or current, *via* strip chart recorder or integrator	Requires calibration at time of analysis
Enzymatic oxidation with alcohol dehydrogenase	Dilution (for plasma or serum) or deproteinization	Selective for ethanol; some interference by isopropanol and methanol	Ultraviolet spectrophotometer, visible photometer, or some automatic analyzers	Photometric/ spectrophotometric reading	Potential interference by higher alcohols, some enzyme inhibitors
Chemical oxidation with acid dichromate	Distillation, diffusion, dialysis, or aeration	None	Distillation or diffusion apparatus, photometer or spectrophotometer, or titration device, water bath	Titration or photometric/ spectrophotometric reading	Time consuming; nonspecific
Osmometry	None for plasma or serum	None	Freezing-point depression osmometer	Osmolality measurement	Nonspecific; only plasma or serum specimens usable; poor correlation with blood-alcohol concentration

complete specificity for ethanol is achieved by using multiple columns and analysis conditions. Analysis of the "headspace" vapor above a blood or other liquid specimen saturated with sodium chloride, after equilibration at 50 C or other controlled temperature, is a simple and desirable gas chromatographic technic. It is usually preferable to direct injection into the chromatograph of a diluted whole blood specimen because it obviates problems of inlet and column contamination and syringe plugging. Direct injection of a diluted liquid sample,[5] however, may be preferable for emergency tests because it eliminates the 15–30-min headspace equilibration period. When many blood specimens are to be analyzed for alcohol and analyst time is limited, automated gas chromatographic headspace analysis[10] or the use of automatic liquid sampling attachments is indicated and yields excellent results in routine use. The principal variants of gas chromatographic methods for blood-alcohol analysis are listed in Table 2 in order of the author's preference. As indicated in Table 1, gas chromatography requires the use of simultaneous reference standards.

Table 2. Principal Variants of Gas Chromatographic Methods for Blood-alcohol Analysis

- Detectors
  - Flame-ionization detector
  - Thermistor detector
- Appropriate packed columns
  - Solid phase (Porapak®, Chromosorb®, *etc.*)
  - Liquid phase (Carbowax®, Hallcomid®, *etc.*)
- Analysis technics
  - Headspace sampling after equilibration
  - Direct injection of diluted blood
  - Protein precipitation and direct injection
  - Options: internal standard *vs.* direct procedures
- Quantitation
  - Electronic integration/printout of peak areas
  - Measurement of strip-chart recording of detector response: peak heights or peak areas

Enzymatic oxidation with alcohol dehydrogenase (ADH) is a sensitive and simple method for alcohol measurement. It is a practical method for occasional or infrequent use because elaborate preparation is not needed, and for the same reason, it serves well as a back-up procedure for gas chromatography. The two principal variations are measurement of the change in ultraviolet absorbance at 260 or 340 nm, and visual photometry of a secondary indicator reaction. Several commercial kits are available for ADH methods; the characteristics and performances of four of these were reported by Redetzki and Dees.[15] The principal features of the ADH methods are given in Table 3. Several instrumental variations have been reported, including the use of rapid centrifugal analyzers,[11] rapid electrochemical measurement using a membrane oxygen-sensing electrode,[3] and adaptation to the DuPont Automatic Clinical Analyzer®.[1] All ADH-based enzymatic

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Table 3. Principal Features of Enzymatic (ADH) Oxidation Methods for Blood-alcohol Analysis

Reactions

Basic Reaction: $C_2H_5OH + NAD^+ \overset{ADH}{\rightleftharpoons} CH_3CHO + NADH + H^+$

Conditions: *p*H 8.7–9.6; CH_3CHO trapped with semicarbazide or aminoacetic acid

Diaphorase: $INT + NADH + H^+ \overset{Diaphorase}{\rightleftharpoons}$ Red Formazan $+ NAD^+$

Oxygen Depletion: $NADH + H^+ + \frac{1}{2}O_2 \xrightarrow[Mn^{++}]{Peroxidase} NAD^+ + H_2O$

Catalyst
NAD oxidoreductase (alcohol dehydrogenase; EC 1.1.1.1)

Preparation of plasma, serum, or blood
Dilution with saline solution or deproteinization with perchloric or trichloracetic acid or $Ba(OH)_2 + ZnSO_4$

Final measurement
Ultraviolet spectrophotometry
Δ Absorbance at 260 nm (NAD^+ absorbs)
Δ Absorbance at 340 nm (NADH absorbs)
Visible photometry: measurement of stable red formazan color at 500 nm
Amperometric measurement with O_2 electrode
Fluorometry

oxidation methods are subject to potential interference by methanol and, especially, isopropanol to varying extents.[15,16] Reference standards must be analyzed in parallel with the biologic samples and controls, as they should be irrespective of the alcohol analysis method employed.

In view of the advantages of other methods, chemical oxidation with potassium dichromate in sulfuric (or nitric) acid solution is now one of the less preferable methods. It requires substantial sample treatment and separation of the alcohol from its matrix before reaction with the acid-dichromate reagent. It is time-consuming in the usual method variations, in addition to being essentially nonspecific for ethanol unless combined with complex chemical manipulations. Stability of the reagent and simplicity of the final measurement do not sufficiently counterbalance these disadvantages for use in the general clinical laboratory. The method may be useful for back-up purposes for the gas chromatographic or ADH methods, since a negative result conclusively eliminates the presence of alcohol in the specimen, and potential chemical interferants can be detected by simple tests on the same distillate.[4]

Table 4 correlates BACs with alcoholic influence stages and the corresponding clinical signs and symptoms. Great caution should be exercised in correlating BACs with presumed alcohol dosage, and speculative retrograde extrapolation of an experimentally determined BAC to an earlier time should be avoided because of its many pitfalls.[8]

Table 4. Stages of Acute Alcoholic Influence/Intoxication

Blood-alcohol Concentration (% w/v)	Stage of Alcohol Influence	Clinical Signs/Symptoms
0.01–0.05	Sobriety	No apparent influence Behavior nearly normal by ordinary observation Slight changes detectable by special tests
0.03–0.12	Euphoria	Mild euphoria, sociability, talkativeness Increased self-confidence; decreased inhibitions Diminution of attention, judgment, and control Loss of efficiency in finer performance tests
0.09–0.25	Excitement	Emotional instability; decreased inhibitions Loss of critical judgment Impairment of memory and comprehension Decreased sensitory response; increased reaction time Some muscular incoordination
0.18–0.30	Confusion	Disorientation, mental confusion; dizziness Exaggerated emotional states (fear, anger, grief, *etc.*) Disturbance of sensation (diplopia, *etc.*) and of perception of color, form, motion, dimensions Decreased pain sense Impaired balance; muscular incoordination; staggering gait, slurred speech
0.27–0.40	Stupor	Apathy; general inertia, approaching paralysis Markedly decreased response to stimuli Marked muscular incoordination; inability to stand or walk Vomiting; incontinence of urine and feces Impaired consciousness; sleep or stupor
0.35–0.50	Coma	Complete unconsciousness; coma; anesthesia Depressed or abolished reflexes Subnormal temperature Incontinence of urine and feces Embarrassment of circulation and respiration Possible death
0.45+	Death	Death from respiratory paralysis

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