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Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline

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Foreword

This guideline, *Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline*, was developed in response to the frequently expressed need for a readily available information source which addresses the increasingly more frequent involvement of the hospital or independent clinical laboratory in collecting and analyzing blood (and other biological specimens) for ethyl alcohol. The presence of alcohol is frequently associated with trauma, and with a great variety of acute illnesses and chronic diseases. Further, alcohol presence often adversely affects both morbidity and mortality. Therefore, appropriate trauma care and diagnosis and treatment of many medical syndromes and diseases in adults require information about the patient's alcohol status. Demand for blood alcohol testing of patients can, therefore, be expected to continue to increase.

Years of experience have borne out the expectation that the absence, or presence and concentration of alcohol in blood will often have later medicolegal or forensic implications and significance, in addition to its immediate clinical relevance. Some simple and practical measures taken at the outset can greatly reduce the impact of such subsequent legal developments on the clinical laboratory and its personnel. This guideline addresses that issue and the resultant responsibilities of clinical laboratories which are not limited to, but include the collection of blood specimens, quality assurance, records, and reports.

The Subcommittee on Blood Alcohol Testing has endeavored to produce a brief but adequate set of criteria to assist clinical laboratories in meeting the demand for timely and reliable blood alcohol testing for clinical purposes, while minimizing the impact of later medicolegal developments on the laboratory.

Universal Precautions

Because it is often impossible to know which might be infectious, all patient blood specimens are to be treated with "universal precautions." Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention. NCCLS document M29, *Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*, deals specifically with all aspects of this issue.

Key Words

Alcohol, blood alcohol, analysis, blood alcohol concentration, chain-of-custody, intoxication.

Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline

1 Scope and Requirements

1.1 Scope of the Problem

The cost of alcohol abuse in our society is extraordinarily high in terms of the loss of human life, its detrimental contribution to the causes of illness and injury, productivity losses in the workplace, and the stress these in turn put on our medical resources and our judicial system. In a series of special workshops on alcoholism and alcohol abuse, the American Society of Clinical Pathologists Task Force on Drug Abuse and Toxicology¹ recognized the magnitude of the problem of alcohol in our society. The title of these seminars was "Alcohol - The Second Great Imitator" because of the medical challenges in the diagnosis of this problem.

1.2 Medical Requirements

Alcohol abuse should be considered one of the most important contributory causes of injury and disease today. The diagnosis of alcohol-use disorders, such as alcohol dependence and alcohol abuse, is a clinical procedure and subject to the problem of inexactness.² Diagnosis of the disease may be based not only on the features of alcoholism, with all its signs and symptoms, but on an accurate determination of blood alcohol concentration at the time of examination of a patient.

The use of alcohol acutely affects the central nervous system. Many of the signs and symptoms manifested by the patient are related to the degree of intoxication, as reflected by the concentration of alcohol in the patient's blood. Since many patients with traumatic injuries who are admitted to the emergency department are noncomatose, patient history or initial interview becomes most important. Patients showing direct effects of alcohol—breath odor, released inhibitions, alcoholic facies, toxic amblyopia, possible tachycardia and cardiac arrhythmias, nystagmus, and traumatic injuries of varying degrees—warrant further studies. These studies should include a complete physical examination, clinical laboratory analyses, and determination of the blood or serum alcohol concentration. Other drugs of misuse or abuse

should also be considered in the testing procedures. The information in Dubowski's table on the Stages of Acute Alcoholic Influence/Intoxication³ (Appendix A) and the Guide to Serum-Alcohol Test Results (Appendix B) become important in the evaluation of the patient, for they define possible correlations of blood alcohol concentration with its clinical signs and symptoms.

Because alcoholism can masquerade as many other diseases, vital signs become very important during the physical examination, and the possibility of closed head injury or neurological disorder should be considered. Associated disease diagnoses include neurological disorders; alcohol-induced or nonalcohol-associated cardiovascular disorders; arrhythmias, tachycardias, electrocardiographic alterations; liver disease; fatty liver, alcoholic hepatitis, portal fibrosis, cirrhosis and possible liver carcinoma; blood and blood clotting disorders: anemia, prothrombin elevations and thrombocytopenia; alcoholic pancreatitis; infections; alcoholic myopathies; digestive tract disorders: ulcers, gastritis, esophagitis, esophageal varices and cancer; endocrine disorders; skeletal system disorders: ischemic necrosis of the femoral heads and fractures; skin disorders; and toxic psychoses.

Indicated laboratory studies include analysis for blood alcohol concentration and other relevant clinical laboratory tests.

The evaluation of the *comatose patient* may be more difficult due to the lack of patient history. The physical examination and results of laboratory studies often reveal the diagnosis. These should be combined with radiological studies, particularly of the skull and chest. A proper evaluation of these studies will be valuable for decisions regarding admission to the hospital, proper treatment, and to minimize possible medical and legal complications.

This document necessarily emphasizes certain legal and other nonclinical aspects of blood alcohol testing. It is, therefore, important to recognize at all times that the clinical laboratory's first and primary responsibility is to the patient and to the physician.

1.3 Industrial-Medical Requirements

Alcohol is the most commonly used drug in the United States. It accounts for industrial losses in billions of dollars. For example, productivity losses due to alcohol abuse were estimated at \$27 billion in 1985, or 39 percent of the total economic cost of alcohol abuse for that year.⁴ Because of the recognition of the economic cost due to alcoholism and alcohol-related injuries, blood alcohol determinations may be required in connection with the following:

- Employment-related (on-the-job) injury
- Workers compensation (federal, state, local) proceedings
- Employee insurance programs
- Pre-employment screening and evaluations
- Employee drug screening
- Alcoholism treatment programs.

1.4 Medicolegal Requirements

Most blood-alcohol analyses in hospitals and other clinical settings are performed solely for medical diagnostic, treatment-related, or other clinical purposes. In such clinical laboratories there is no requirement for chain-of-custody procedures (see Section 3). In many jurisdictions the results of alcohol analyses may ultimately become evidence in civil or criminal legal proceedings, regardless of their original purpose. There is, however, no rational basis for the mystery and trepidation with which alcohol analyses are often regarded in clinical laboratories.⁵ Some simple practices can minimize the extent of involvement of clinical laboratory personnel in subsequent legal proceedings, with respect to the collection and analysis of blood for alcohol for clinical purposes. A frequently updated, comprehensive, written (and/or computerized) protocol which is adhered to as necessary—minimizing the number of people involved in collection, transport, analysis for alcohol, and storage of a given blood specimen; as well as a clear statement on all records and reports that the specimen analyzed was whole blood, serum, or plasma, etc., can significantly reduce the involvement of clinical laboratory personnel in subsequent legal proceedings.

In some institutions, the analysis (or collection only) of blood specimens for alcohol for

exclusively legal purposes such as traffic law enforcement or for potential adversarial proceedings such as accident-related workplace alcohol testing⁶ is a regular occurrence. In those circumstances, the laboratory can establish a two-option system, in which the full legal requirements such as chain-of-custody procedures and specimen seals and secure specimen storage are reserved for legal-category tests. The investigation of a medico-legal case and the interpretation of the results of analysis in the legal context require good judgment and the assurance that the specimen has been properly collected and processed. If the following factors are appropriately handled, the laboratorian will have far fewer problems with testimony or legal proceedings in a court of law.

1.4.1 Consent

This problem can be dealt with through statutory provisions such as implied consent laws in each jurisdiction, or through legal advice regarding individual consent to obtaining a sample.

1.4.2 Collection Techniques

Knowledge of the statutes of the jurisdiction concerned and implementing national or regional regulations are necessary, for these authorities may designate who may draw the sample, the specimen container to be used, and how it is preserved.

1.4.3 Identification Procedures

For both medical and subsequent legal purposes, if any, a foundational requirement is to establish from whom the blood specimen was collected, by whom, at what date and time, etc. These data provide for "traceability" of the specimen—an aspect as important as specimen integrity (unaltered state).

1.4.4 Chain-of-Custody

This is the documentation discussed in Section 3 that accompanies the specimen if chain-of-custody procedures are used in a given instance. It certifies that: the specimen was obtained from the individual named as the source of the specimen, the specified laboratory was responsible for the analysis, all individuals who had possession of the specimen

prior to analysis are listed, as well as the name of the technician who performed the analysis.

1.4.5 Security System

This must be utilized, when appropriate, to maintain the chain of custody and to exclude the possibility of tampering.

1.4.6 Method of Analysis

This must be a recognized method having the requisite reliability, and it must be accompanied by adequate quality assurance procedures. See Section 4.

1.4.7 Interpretations

There are many requirements for interpretation of blood alcohol concentrations and they are both medical and legal in nature. Many of the problems presented in this overview will be addressed in other parts of this document. Certain aspects, however, such as extrapolation of alcohol test results to other times, are beyond its scope.

1.4.8 Checklist of Issues

A checklist of issues that routinely surface during legal proceedings involving the analysis and reported results of a blood alcohol specimen appears as Table 1.

2 Specimen Collection

Recent revisions of motor vehicle codes in many states, in order to combat the problem of driving under the influence of alcohol (DUI) or while intoxicated (DWI) have placed additional responsibilities on hospitals and clinical laboratories. These revisions have created a need for guidance concerning the collection and processing of specimens for blood alcohol analysis. The discussion which follows is intended to assist phlebotomists and laboratory personnel involved with the collection and processing of specimens for alcohol determination.

2.1 Responsibility for Specimen Collection

Several important considerations are involved in decisions made by medical personnel to collect blood specimens from traffic accident victims for alcohol analysis. From a medical treatment viewpoint, it may be desirable, or necessary, to know the patient's blood alcohol concentration before administering anesthetics or medications. Such test results may also be utilized in later legal proceedings to determine whether the person was intoxicated or under the influence of alcohol.

2.2 Types of Specimens

Plasma and serum are physiologically and pharmacologically more appropriate specimens than whole blood. Intravascular alcohol transport involves both the cellular and noncellular components of blood, but alcohol distribution occurs primarily between the circulating plasma and other body tissues and fluids.

Motor Vehicle Codes or other laws may state that breath, blood, or urine may be analyzed for alcohol content. Of these fluids, blood, serum, or plasma are usually analyzed in clinical laboratories, since breath-alcohol testing equipment is generally not available in the clinical laboratory, and since urine alcohol concentrations are not well correlated with blood alcohol concentrations.^{5,7,8}

When the term "blood" is used in motor vehicle statutes, *whole blood* is the universal meaning. Most regional laws define the alcohol element of drinking/driving offenses wholly or partly in terms of blood-alcohol concentrations; and may specify whole blood as the required specimen when "blood" is analyzed. Hence, if blood rather than breath is to be analyzed for alcohol, either exclusively or primarily in connection with traffic law enforcement, it is best to analyze whole blood.

Table 1. Checklist of Issues Commonly Arising in Legal Proceedings Involving Blood-Alcohol Analysis Results

Laboratory and Personnel

- Identity, status, and qualifications of the person giving testimony
- Name, status, and location of laboratory
- Identity, status, and qualifications of the analyst(s) and phlebotomist
- Identity, status and qualifications of the laboratory director
- Licensure and accreditation of laboratory and personnel

Specimens

- Information and documentation on identity of the specimen donor; and on the nature, integrity, and security of specimens
- Authority for ordering the test and for procurement of the specimens
- Appropriateness and validity of specimen selection
- Details of specimen collection, handling, storage
- Time, date, location of specimen collection; and bodily sampling site
- Chain-of-custody of specimens, and evidence to establish absence of irregularities or tampering; seals, labels, etc.
- Details of specimen containers, anticoagulants, preservatives, etc.
- Present location and condition of specimens or residues

Analysis

- Analysis protocols and standard operating procedures; literature references
- Details of the analysis actually performed; including apparatus, equipment, devices, and procedures involved
- Analytical "raw" data and final results and findings, and their derivation
- Details of instrument or system calibration
- Method characteristics, especially with respect to accuracy, precision, linearity, sensitivity, specificity, interferences
- Validity and reliability of the analysis scheme

Quality Assurance

- Overall quality assurance/quality control schemes
- Control specimens; nominal concentrations, actual results
- Standards, and their origin and validation
- Replicate analyses of the unknown specimens
- Laboratory performance in external alcohol analysis proficiency testing programs and surveys

Interpretation of Results

- Scientific validity of the test results
- Pharmacological, toxicological, or clinical significance and meaning of the results
- Relevance or significance of the results to the legal issues
- Relevant patient history, clinical care and treatment details; effects on results of fluid(s) administration, medications, shock, trauma or other details of patient status or medical conditions
- Compliance with applicable statute law, case law decisions, and rules and administrative procedures

Documentation

- Records, laboratory requisitions and requests, reports, manufacturers' literature and other source documents pertaining to any of the foregoing matters

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Alcohol, at equilibrium, is generally distributed throughout the body in proportion to the water content of various fluids, tissues, and organs. In particular, the alcohol concentration of whole blood is not identical to that of plasma or of serum. However, the alcohol concentration of either serum or plasma is, in practice, the same. Both theoretical calculations, based on water content, and experimental data yield typical mean ratios of 1.12/1 to 1.18/1 in normal subjects for serum/whole blood alcohol concentrations, with typical experimental ranges of 1.05/1 to 1.25/1.^{9,10}

The specimen type analyzed should be identified. Results of alcohol analysis on serum or plasma specimens should not be converted to whole blood concentrations. If courts require the interpretation of serum alcohol concentrations or the conversion of serum concentrations to whole blood concentrations, experts can be retained to perform these functions. It is a complex issue.¹¹

2.3 Specimen Collection, Handling, and Preservation

The blood collection procedure for *forensic* alcohol determinations must be conducted so that no doubt exists as to the authenticity and validity of the specimen. In this regard, several points should be emphasized.

2.3.1 Time of Collection

The time of collection is critical information which must be recorded and should appear on the report of results.

2.3.2 Site of Venipuncture

The site of the venipuncture is usually the median cubital or one of the other superficial veins of the forearm. Veins in the lower extremities can also be used if the forearms are not accessible because of injuries or for other reasons. During the early phases of alcohol absorption, peripheral venous blood concentrations lag behind arterial blood concentrations, particularly in the lower extremities.

Blood should not be removed from veins into which intravenous fluids or other medications are being administered at the time. The dilution effect can lower the alcohol concentration.

Even when the presence of such parenteral fluids would not be expected to significantly affect the alcohol concentration of the blood, it is better to select a venipuncture site remote from the location of fluid administration in order to ensure a specimen representative of the true alcohol concentration of the specimen. If possible, it is best to collect the specimen before any treatment is begun. Ideally, venipuncture should be performed in accordance with applicable procedures described in the NCCLS document H3, *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture*.¹²

2.3.3 Disinfectant

The disinfectant used for cleansing the venipuncture site should not contain alcohol or other volatile organic substances. The most frequently employed disinfectants for this purpose are aqueous benzalkonium chloride or aqueous povidone-iodine.¹³ Studies by Dubowski and Essary¹³ have revealed that blood specimens can be significantly contaminated if alcohol containing sponges are used to cover the venipuncture site at the time when the needle is withdrawn from the vein while attached to the vacuum tube. Therefore, to avoid the possibility of contamination and legal challenges to the acceptability of the specimen collection procedure, only nonalcoholic disinfectants should be employed, and sterile dry sponges should be used to cover the venipuncture site. Further, if evacuated collection tubes are used, the tube should be removed from the multisample collection needle and holder before withdrawing the needle from the puncture site.

2.3.4 Specimen Container

The specimen container is important and will vary depending on whether serum, plasma, or whole blood is to be analyzed. If serum is required, the blood should be collected in a container without a preservative or anticoagulant and allowed to clot. The serum can be sent directly to the laboratory without further processing if the specimen is to be analyzed for alcohol content within four hours. If the analysis will be delayed, the serum should be transferred to another container and treated with sufficient sodium fluoride to produce a minimum concentration of 10 mg/mL (0.24mmol/ml).

For whole blood or plasma specimens, the type and amount of anticoagulant present is not important if the specimen is analyzed within four hours of collection. It is only necessary that the anticoagulant not interfere with the alcohol determination and that a sufficient quantity is present in the specimen to prevent clotting. If the analysis is to be delayed, additional safeguards must be instituted to prevent changes in the alcohol content of the blood. For this purpose, potassium oxalate monohydrate (5 mg/mL of blood; $2.7\mu\text{mol/mL}$) and sodium fluoride (1.5 mg/mL of blood; $3.6\mu\text{mol/mL}$) are an appropriate anticoagulant and preservative combination for storage at 5 °C of initially sterile blood specimens for up to 48 hours.¹⁴ Blood alcohol specimens stored at -20 °C or below are stable indefinitely.

Specimens that are to be transported or mailed in an unrefrigerated condition, or stored for more than 48 hours should be preserved with higher concentrations of sodium fluoride (10 mg/mL of blood; 0.24mmol/mL).⁸ However, it has been documented that changes produced by contaminating microorganisms can affect alcohol concentrations in blood specimens even in the presence of preservatives. Blume and Lakatua¹⁵ reported that various organisms isolated from contaminated blood specimens were capable of producing ethanol when inoculated into bank blood. *Candida albicans* was particularly active in this regard, producing significant quantities of alcohol even in the presence of sodium fluoride. These investigators recommended that fluoride (10 mg/mL; 0.24mmol/mL) be used as a preservative and that care should be taken to assure that microbial organisms are not introduced into the specimens.

Winek and Paul¹⁶ reported that alcohol analyses of blood obtained under sterile conditions from living humans can be delayed as long as 14 days without a significant change in alcohol content. They state that this holds true whether the blood sample is refrigerated or not, or whether a preservative is added to the sample. Nevertheless, the question can still arise as to how the phlebotomist could know with certainty, even if aseptic collection techniques were employed, that no microorganisms entered the specimen and produced changes in the alcohol concentration. For this reason, it is advisable to employ preservatives

and to refrigerate specimens as additional safeguards against changes in alcohol content.

2.3.5 Size of Sample

The size of the sample should be sufficient to permit retesting, if necessary.

2.4 Specimen Handling

To ensure complete dissolution of the fluoride in the blood, the closed container of blood should be gently inverted several times immediately following specimen collection.

The laboratory request form for alcohol analysis should be completed legibly and should contain the following information:

- Patient's full name
- Identification number
- Time and date of specimen collection
- Site of venipuncture
- Phlebotomist's name
- Name and address of facility where specimens were collected.

Collection kits designed to facilitate the sampling process are available from commercial sources. Potential purchasers should determine that the kits meet their needs and comply with local laws concerning blood alcohol analysis.

Additional special specimen handling considerations are addressed in Appendix C.

2.5 Replicate Blood Specimens

When it is known at the outset that alcohol analysis results will be required for legal purposes as well as for immediate clinical patient care, it may be practical and appropriate to collect replicate blood specimens in parallel and with consideration for the required kind of specimen, e.g., unpreserved serum for immediate analysis for clinical purposes, and a preserved anticoagulated whole-blood specimen for separate medicolegal analysis. Different documentation and handling may be required in such instances.

3 Chain-of-Custody Procedures^{17,18}

3.1 Purpose

The established relationships between alcohol, trauma, and litigation ensure that the results of many medically-indicated blood-alcohol analyses performed in hospital and other clinical laboratories will later be sought as evidence in civil or criminal litigation or other adversarial proceedings, such as formal arbitration. (Introduction of such evidence demands a requisite degree of proof; an important element of that proof is the chain-of-custody.) In addition, alcohol analysis is frequently sought exclusively for legal reasons, such as in drinking-driving investigations. The goal is to provide adequate and acceptable proof of the identity of the specimen and specimen donor, to assure the integrity of the specimen throughout its existence, and to eliminate significant changes in the specimen composition before analysis.

The laboratory's responsibility for initiating and maintaining the chain-of-custody begins when the specimen is collected (if by that laboratory's personnel) or when the specimen first reaches the laboratory. The laboratory's responsibility ends when the specimen is entirely consumed in its analysis, or when custody of the specimen is transferred to any party outside of the laboratory, or if the specimen is destroyed on proper authority. In the first event (specimen consumption), the laboratory's chain-of-custody responsibilities continue with respect to the original specimen container.

Chain-of-custody, in the context of this document, is a term-of-art pertaining to the procedures and documentation necessary for legal purposes to establish the identity and origin of the blood specimen; to enable it to be traced to the person from whom it was collected; to reasonably assure absence of specimen mix-up; deliberate adulteration; or other tampering; to exclude unauthorized access at any stage of specimen collection, storage, analysis and retention; as well as to prevent loss of the specimen. Such proof is required in part because blood is fungible—meaning that blood specimens typically cannot be visually distinguished from each other, and because alteration or substitution

could readily occur in the absence of proper safeguards. The basic concept of the chain-of-custody is to trace a specimen, step-by-step, and person-by-person, from its origin to the completion of the analysis (and beyond if further retained). The chain-of-custody must document the location and care of the specimen at all times by identifying sequentially every person having responsibility for and control of the specimen for any purpose. Every change in custody must be documented by appropriate entries into a dedicated record. The concept of the chain-of-custody and the practices associated with it originated long ago in the collection and preservation of physical evidence and its subsequent admission in litigation. These practices have become widespread and familiar to clinical laboratories especially in connection with the recent large scale drug-use testing of biological specimens.

3.2 Documentation

Laboratories which routinely undertake blood alcohol testing should anticipate being required to provide chain-of-custody information and documentation, sometimes months or years after the testing date. The obvious choices are to establish, maintain, and document the chain-of-custody for all blood-alcohol specimens/tests, or to do so only in selected predetermined instances of the kind most likely to be involved in later litigation, such as accidents, assaults, trauma, workplace-related events, etc. Clearly, blood alcohol testing originally conducted under legal mandate, e.g., police-directed or court-ordered tests of arrested drivers, should always involve the full chain-of-custody scheme.

Several principles apply to chain-of-custody procedures and their documentation with special purpose forms. Both the procedures and their documentation should be simple, limited to essentials, easy to use, and readily traceable, with minimal reliance on testimony of the people involved with a given blood specimen. Access to specimens, at any stage, should be restricted as much as possible. Adequate, unique labeling should establish the identity of the specimen. Physical security measures, such as proper seals, tamper-evident containers, and secure (locked) storage should be employed for all affected specimens.

The chain-of-custody record should contain necessary information on the following elements in logical sequential form:

- (1) Data on the origin of the specimen, as listed in Section 2.4.
- (2) Date, time, location, when relevant, and full identity of all those involved in every transfer of custody or physical possession of the specimen, together with the purpose of, or reason for, each transfer.
- (3) Details of any irregularity, such as container breakage, spillage, or other loss which can affect the specimen.
- (4) Any pertinent remarks bearing on the original identity, integrity, composition, condition, location, or custody of the specimen, (or any change not reflected in other entries).

The entries should be brief and to the point. For example, a temporary transfer of custody may occur when the analyst is absent during a break and the unsealed specimen remains on a laboratory workbench. The required entries in the chain-of-custody document include the date(s) and time(s) and the people involved (from whom-to whom-to whom) in the initial transfer of custody from the analyst to a qualified coworker. The transfer returning custody to the analyst and a statement of reason for each change, such as "transfer due to absence during a break" and "resumption of analysis" must be documented.

Clearly, it is desirable to minimize the number of custody transfers, especially temporary ones, and, hence, the number of chain-of-custody entries. The need for temporary transfers can be largely obviated by arranging for storage of specimens during brief absences in a locked refrigerator compartment, lockbox, locker, container, or secure location accessible only to the custodian.

Chain-of-custody documents can be designed for individual or multiple specimens. In most clinical laboratories, forms designed for use with a single specimen are most useful. The essential information, in addition to the identity and origin data under item 1 above, can be recorded in separate rows for each change of

custody or other transaction, in four columns: the date and time; typed/printed/stamped name of the person releasing the specimen; the identical information for the person receiving the specimen; and the reason for the change. Legibility of all entries, including the signature, is indispensable for such a record. All other hand-written entries should be legibly printed in ink. Whether or not the chain-of-custody document physically accompanies the specimen at all times or is kept in a secure location is up to the laboratory. In most situations, keeping the specimen and a dedicated chain-of-custody document together at all times is preferable.

4 Methods of Analysis

Of the multitude of methods available for the determination of ethyl alcohol (or ethanol) in blood specimens, the two summarized in Table 2 are appropriate for use in clinical laboratories and are listed in the order of preference.¹⁴ A comprehensive review of major developments in alcohol analysis has been published.¹⁹

4.1 Gas Chromatography

Gas chromatographic (GC) determination of ethyl alcohol (or ethanol) analysis can be performed with simple and relatively inexpensive instruments. It yields information on both the identity and concentration of ethanol and other volatile constituents of the sample. Currently, GC is the method of choice. Its desirable features include rapidity, sensitivity, minimal sample preparation and manipulation, and specificity for ethanol. Essentially complete specificity for ethanol can be achieved with gas chromatography. This is accomplished by using multiple columns, by varying analysis conditions, or by performing GC analysis after treating one of two identical sample aliquots with approximately 250 units of alcohol dehydrogenase (EC* 1.1.1.1) per mL of blood or serum. This procedure is followed by gas chromatography; thereby abolishing any ethanol GC "peak" in the treated sample in comparison with the untreated sample, and greatly enhancing the certainty of identification

*EC = Enzyme Commission. In the listing of an enzyme, the abbreviation is followed by four numbers indicating the classification of the enzyme according to main division, subclass, sub-subclass, and serial number of the sub-subclass.

Table 2. 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 equilibrium	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 in high concentrations	Ultraviolet spectrophotometer, visible photometer, or some automatic analyzers	Photometric/spectrophotometric reading	Potential interference by higher alcohols, some enzyme inhibitors, etc.

Modified with permission from Dubowski KM. Alcohol determination in the clinical laboratory. *Am J Clin Path.* 1980;74:747-750

of ethanol. Analysis of "headspace" vapor above a blood or serum sample saturated with sodium chloride, ammonium sulfate, or certain other salts and then equilibrated at 50 °C or other controlled temperature is a simple and desirable gas chromatographic technique.²⁰ Headspace analysis is usually preferable to direct injection of diluted whole blood or serum specimens into the chromatograph inlet because it eliminates problems of inlet and column contamination and syringe plugging. Direct injection of a diluted liquid sample may be preferable for emergency tests because it eliminates the 15-30 minute equilibration period required for headspace analysis. When many blood specimens are to be analyzed, automated gas chromatographic headspace analysis^{21,22} or use of automatic liquid sampling (injection) attachments is indicated. The principal variants of gas chromatographic methods for blood-alcohol analysis are listed in Table 3. Use of internal standards has become the accepted norm.²⁰

4.2 Enzymatic Oxidation with Alcohol Dehydrogenase

Enzymatic oxidation with alcohol dehydrogenase (ADH) or alcohol oxidase (AO) is a sensitive and simple method for alcohol measurement. A variety of both automated and manual methods based on this principle is available for routine use. It is also practical for occasional or infrequent use, because elaborate preparation is not necessary, and reliable single-assay reagents are commercially available. For the same reasons, it serves well as a back-up method for gas chromatography. The principal variants are use of alcohol oxidase or alcohol dehydrogenase, measurement of the change in ultraviolet absorbance of the reaction mixture at 260 or 340 nm, or visual photometry of a secondary indicator reaction. A variety of commercial reagents and kits are available for ADH methods. The characteristics and performance of three ADH methods have been reported.^{23,24}

The principal features of ADH methods are given in Table 4.

Table 3. Principal Variants of Gas Chromatographic Methods for Blood-Alcohol Analysis**Detector**

Flame-ionization detector

Appropriate packed columns

Solid phase (Porapak and Chromosorb, are examples of commonly used solid phase columns)*

Liquid phase (Carbowax and Hallcomid are examples of commonly used liquid phase columns)*

*Other suitable columns may be substituted.

Appropriate capillary columns

Large bore

Liquid-coated walls

Analysis techniques

Headspace sampling after equilibration

Direct injection of diluted blood or serum

Protein precipitation and direct injection of the supernatant liquid

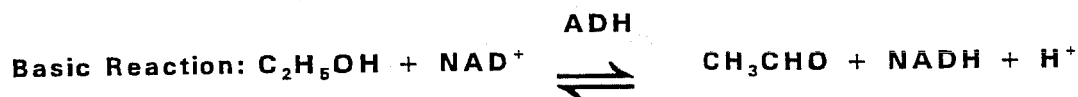
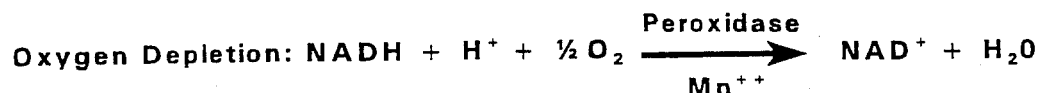
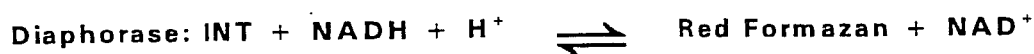
Internal standard added to sample

Quantitation

Electronic integration/printout of peak areas

Dedicated electronic controllers/data systems

Measurement of strip-chart recording of detector response: Peak heights or peak areas

Modified with permission from Dubowski KM. Alcohol determination in the clinical laboratory. *Am J Clin Path.* 1980;74:747-750**Table 4. Principal Features of Enzymatic (ADH) Oxidation Methods for Blood-Alcohol Analysis****Reactions**Conditions: pH 8.7-9.6: CH₃CHO trapped with semicarbazide or aminoacetic acid**Diaphorase****Catalyst**

NAD oxidoreductase (alcohol dehydrogenase: EC 1.1.1.1)

Preparation of plasma, serum, or blood

Dilution with saline solution or deproteinization with perchloric acid or trichloroacetic acid or Ba(OH)₂+ ZnSO₄**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 O₂ electrode

Fluorometry

Modified with permission from Dubowski KM. Alcohol determination in the clinical laboratory. *Am J Clin Path.* 1980;74:747-750.

There is considerable variation in the types of instruments employed in these procedures. Some procedures use rapid centrifugal analyzers, while others employ rapid electrochemical measurement using immobilized ADH and AO enzymes and oxygen-sensing electrodes. Other methods have been adapted to automated analyzers, or use discrete sample analysis employing radiative energy attenuation with ADH, based on the principle of fluorescence quenching. ADH-based enzymatic oxidation methods are subject to potential interference by elevated concentrations of isopropanol and higher alcohols to varying extents, while AO-based methods cannot distinguish between ethanol and methanol. At commonly encountered concentrations of isopropanol and methanol, ADH-based methods are not significantly affected.

Details of a well-documented enzymatic oxidation procedure are given in Gadsden and Taylor in "Ethanol in *Biological Fluids by Enzymic Analysis*." (*Selected Methods of Emergency Toxicology*. Washington, DC, AACC Press, 1986;63-65.)

5 Quality Assurance

The general recommendations for quality assurance which follow exemplify good laboratory practice standards as applied to blood alcohol testing. Because clinical laboratory procedures are continually evolving technologically, it is important to integrate into the laboratory's good laboratory practices, as a minimum, directions for test performance, establishment and validation of calibrations, checks on linearity, and other analysis instructions provided by the applicable manufacturer(s) of the instrument(s) and commercial reagents utilized. Further, the ongoing mandates on use of controls and other good laboratory practices promulgated by regulatory and other applicable authority should be recognized and complied with when applicable.

In the discussion on calibrators and controls which follows, separate consideration is given to certain aspects applicable to GC or ADH methods, respectively, to the extent that they differ.

The laboratory should also follow, to the extent feasible, the principles and practices outlined in these NCCLS documents:

- (1) NCCLS Document C24: Internal Quality Control Testing: Principles and Definitions.
- (2) NCCLS Document EP10: Preliminary Evaluation of Quantitative Clinical Laboratory Methods.

5.1 Calibrators (Standards)

The calibrators should be selected to represent critical concentrations, which span the clinically and forensically relevant alcohol concentrations and include the upper limit of linearity of the analysis. These calibrators will bracket the majority of positive results and can be used to demonstrate linearity, in conjunction with a specimen established to be free of alcohol.

Calibrators can be prepared from a freshly opened container of pure ethanol, from a tightly sealed container of stock solution prepared by diluting an aliquot from a freshly opened container of pure ethanol, or from analytical grade 95% ethanol. Calibrators at various concentrations are available from commercial sources. Aqueous Standard Reference Materials containing ethanol are available from the National Institute of Standards and Technology (SRM 1828a). Aqueous standard solutions are also available from the College of American Pathologists, and other sources.

Calibrators contain a designated mass (weight) of ethanol per volume of solution. Technicians preparing standards should take into account that the density of ethanol is less than 1 and varies with temperature.

Gas Chromatography: Every alcohol analysis or batch of analyses performed by GC methods should begin with the analysis of at least one, and preferably two or more different calibrators together with an alcohol-free "blank," because the operating parameters and calibration of GC instruments vary with each startup and can also drift during prolonged operation.

Enzymatic Oxidation: The instrument manufacturer's recommendations for calibration should be followed. Most current generation

analyzers have been shown to have stable calibrations over time, often for months. Well-characterized, stable controls should be included in each analysis or run to verify the validity of such historical calibration. For integrated analysis systems, it is important to use the calibrators provided by the instrument manufacturer, and to avoid using unvalidated calibrators from other sources which may introduce analysis bias as the result of matrix effects.

5.2 Controls

Every analysis or batch of analyses should be accompanied by the analysis of negative and positive controls. The controls should consist of known, appropriate concentrations of ethanol in the same biological matrix as the specimens to be analyzed. Each control should be processed through all steps of the procedure, exactly as each specimen is processed. Concentrations of multiconcentration controls should be targeted at or near decision points such as "cut-offs" separating positive from negative results and near the limit of linearity.

Controls can be prepared by adding known quantities of ethanol to homogeneous preserved biological specimens, mixing thoroughly, and freezing aliquots in tightly sealed containers. Prepared controls for serum and whole blood ethanol (alone or with other alcohols) are commercially available.

Target values and limits for laboratory-prepared controls should be established as described in NCCLS publication C24, *Internal Quality Control Testing: Principles and Definitions*.

If only one specimen is to be analyzed, the standard(s) (if needed) should precede and the control(s) should follow or bracket the specimen.

Each day's quality assurance results should be recorded and compared to previous results to facilitate early detection of changes. The principal purpose of including controls in an analytical run is to determine whether the analytical method at the time is yielding acceptable results. Out-of-control results require immediate action to investigate and correct the analysis performance. Pending such action, blood-alcohol testing should be suspended.

Gas Chromatography: Because of the variability of instrument parameters and calibration with each startup, and the tendency of these factors to drift during prolonged instrument operation, at least every tenth specimen should be a control when multiple, sequential analyses are conducted. Multilevel controls are preferred.

6 Reporting and Significance of Results

6.1 Blood Alcohol Records

Information concerning the specimen for alcohol analysis can be recorded in a bound ledger-type record book with numbered pages which is maintained in the laboratory. Alternatively, recordkeeping can be computerized and often is in the modern clinical laboratory. The same principles apply when computer laboratory information systems are used. Specimen records should include:

- Specimen number
- Patient's name
- Date and time of specimen receipt
- Condition of seals on container and specimen tubes
- Condition of specimen
- Other pertinent information
- Date and time of analysis
- Name of analyst
- Blood (or serum if applicable) alcohol concentration

To shorten the chain-of-custody, if used, it is best if one person processes the specimen from receipt in the laboratory through conducting the alcohol analysis and reporting results.

A file should also be established in which all information relating to the specimen and its analysis is maintained. These files may be organized either alphabetically using the patient's last name, or numerically, using the identification number on the report form. If a numerical system is selected, an alphabetical cross reference index should be established. In either event, it should be possible to access the necessary information chronologically, by the patient's name, and by the specimen identification number. Any information which cannot be conveniently placed directly into this file, should be photocopied, if possible, and included with the other records. This is fre-

quently necessary when information is recorded directly onto the shipping container. Items which cannot be copied should be recorded in the section provided for other information in the bound record book. The source of the items which are copied or transcribed should be clearly specified so that upon subsequent examination, the material can be easily identified.

All records of forensic blood alcohol analyses should be stored in a secure location, with access limited to authorized personnel, for a defined period of time (e.g., ten years). Since civil suits resulting from traffic accidents often take place long after the alcohol analysis, it is not uncommon to have to testify about determinations that were performed two to five years previously and occasionally, even earlier. For this reason, it is important to preserve such records if the laboratory changes ownership or discontinues operations.

6.2 Conversions

The following conversions are frequently used in blood alcohol testing.

g/L	g/dL	% w/v	mg/dL	mmol/L
1.00	0.10	0.10	100	21.71

Conversion factors: $\text{mg/dL} \times 0.2171 = \text{mmol/L}$
 $\text{mmol/L} \times 4.61 = \text{mg/dL}$

7 Terminology and Statutory Provisions

7.1 Terminology

7.1.1 Alcohol

Alcohol refers to ethanol which may carry synonyms of ethyl alcohol, EtOH. If another alcohol is present, it must be specifically identified and uniquely analyzed.

7.1.2 Blood Alcohol Concentration (BAC)

The concentration of ethyl alcohol in whole blood is expressed most commonly as percent by weight/volume (% w/v) or as grams per deciliter (g/dL).

Many regional laws prescribe blood alcohol concentrations constituting impairment or intoxication in terms of percent alcohol "by weight." This means grams of alcohol in 100 mL of blood. The clinical laboratory most commonly determines this by using a measured volume of blood for assay and reporting the alcohol concentration as milligrams of alcohol per 100 mL or dL of blood. The laboratory value may be converted to BAC expressed as percent w/v by moving the decimal point three places to the left. Example: 125 mg/dL converts to 0.125% w/v. However, only two fig-

ures are used in reporting percent. The value is truncated to produce the two digit results, i.e., the third digit is omitted. Example: 139 mg/dL is reported as 0.13% w/v or 0.13 g/dL.

7.1.3 Intoxication

The American Medical Association's Manual²⁵ lists eleven signs and symptoms which are usually accepted as supporting evidence of alcoholic intoxication:

- odor of the breath
- flushing of skin
- loss of muscular coordination
- speech difficulties
- disorderly or unusual conduct
- mental disturbance
- visual disorders
- sleepiness
- muscular tremors
- dizziness
- nausea.

Many of these conditions can result from nonalcohol related pathological conditions. Only careful differential diagnosis, including a determination of alcohol, can distinguish alcoholic intoxication from disease or illness, or show their simultaneous presence.

7.2 Statutory Provisions

ates and provinces have varying definitions of intoxication, impairment, alcoholic influence, and the blood alcohol concentration at or above which it is illegal to operate a motor vehicle. For example, in the United States the laws in general follow the alcohol-related provisions of the Uniform Vehicle Code which have been endorsed by the National Safety Council's Committee on Alcohol and Other Drugs and by the House of Delegates of the American Medical Association.

The following provisions of the UVC²⁶ are widely followed:

1. **Section 11-903(a)5:** Alcohol concentration shall mean either grams of alcohol per 100 milliliters of blood or grams of alcohol per 210 liters of breath (FORMERLY § 11-902(b)4; REVISED, 1979.)

2. **Section 11-903(b)1:** If there was at that time an alcohol concentration less than 0.08, such fact shall not give rise to any presumption that the person was or was not under the influence of alcohol, but such fact may be considered with other competent evidence in determining whether the person was under the influence of alcohol. (REVISED, 1979, 1984; REVISED AND RENUMBERED, 1992.)

3. **Section 11-903(b)2:** If there was at that time an alcohol concentration of 0.08 or more, it shall be presumed that the person was under the influence of alcohol. (REVISED, 1979, 1984; RE-NUMBERED, 1992.)

It should be noted that these provisions apply to motor vehicle operation. There are additional factors that must be taken into account should interpretations be required for other purposes. There is a wide variety of sources for authoritative information regarding the pharmacology and physiological effects²⁷ of alcohol on the human body. Any interpretation of the alcohol concentration is best left to those qualified by experience and training.

The result of a chemical determination of the concentration of alcohol in a person's blood should not be confused with the effect of that alcohol upon the person's brain. The former is measurable with a high degree of accuracy. The latter is not. At each extreme of the BAC (very low or none and very high, 0.40 % w/v or more) there is little doubt about whether the alcohol has had an effect. To set a single concentration, i.e., 0.08 % w/v, as a cut-off point above which each and every person is supposedly affected by alcohol to an equal degree of impairment for all purposes is inappropriate. Some individuals are impaired at less than 0.08 % w/v; others are not obviously impaired at much higher concentrations.

"Under the influence" is a phrase with very wide implications because its significance is not universally agreed upon. One drink may very well produce demonstrable and measurable impairment of judgment and response time. On the other hand, to some people, under the influence means drunk and disorderly and nothing less. Therefore, it is advisable to use other, specific terms to describe impairment for a given task.

Finally, a source of difficulty in equating a blood-alcohol concentration with its effect upon the brain is that the identical blood alcohol concentration(s) can occur in a single individual at two or more different times in a drinking episode. During the absorption of alcohol the patient will pass through various blood alcohol concentrations and again later during elimination of the alcohol will experience them all. To say the patient was equally affected at the two times is unrealistic because of the acute tolerance phenomenon (Mellanby effect) and other factors.²⁸ In many instances, there is greater impairment at a given blood alcohol concentration during the rapid absorption of alcohol than during the elimination phase.

Appendix C. Special Specimen Handling Considerations

Each container of blood should be sealed immediately after the specimen has been drawn and mixed with the preservative. To accomplish this, the phlebotomist should initial the seal, record the date and time on it, and affix it to the stopper and side of the specimen tube in such a manner that the stopper cannot be removed or punctured without disrupting the seal. Paper labels approximately 2 x 2 inches with nonpeelable adhesive on one side are suitable for sealing containers. The specimen container should also be clearly labeled. The label should specify the patient's name and any identification or accession numbers that are needed to relate the specimen to the patient or to the laboratory request form.

If the specimen is to be analyzed in the same facility where it was collected, it should be placed together with the request form and patient consent form in a sealed container and delivered directly to the laboratory where it should be stored in a locked refrigerator until it can be further processed. When the analysis is performed, the person conducting the determination should break the seal on the container and open it to gain access to the forms and specimen.

Specimens to be sent to another facility for analysis should be sealed as described above and either delivered by a messenger or forwarded by First Class Mail (see Related NCCLS Publication H5-A3). Sending specimens by certified or registered mail is recommended to reduce problems with the chain of custody. In a 1951 Nebraska case, the court held that there is a presumption that articles transported by regular U.S. Mail and delivered in the ordinary course of the mails are received in substantially the same condition in which they are sent.¹ The same presumption of regularity and hence lack of a requirement that air shipment couriers or transport agency employees make entries in a chain-of-custody document for a urine sample in transit for workplace drug testing was affirmed in a 1994 decision of the US Court of Appeals for the Eleventh Circuit.² Other court decisions exist to the same effect. If the sample was hand carried and several different people handled it along the way, then technically each person could be subpoenaed to establish a complete chain-of-custody. If the specimen is adequately sealed and forwarded by mail, generally only the person who collected, sealed and mailed the specimen, and the person who broke the seals and analyzed the specimen need testify.

References to Appendix C

1. Schacht vs. State, 50 N.W. 2d 78, 1951.
2. Interstate Brands Corp. vs. Local 441, 10 IER Cases 146-150, 1994.

Appendix A. Stages of Acute Alcoholic Influence/Intoxication (Whole Blood Alcohol)

BLOOD-ALCOHOL CONCENTRATION grams/100 mL.	STAGE OF ALCOHOLIC INFLUENCE	CLINICAL SIGNS/SYMPTOMS
0.01-0.05	Subclinical	Influence/effects not apparent or obvious Behavior nearly normal by ordinary observation Impairment detectable by special tests
0.03-0.12	Euphoria	Mild euphoria, sociability, talkativeness Increased self-confidence; decreased inhibitions Diminution of attention, judgment and control Some sensory-motor impairment Slowed information processing Loss of efficiency in critical performance tests
0.09-0.25	Excitement	Emotional instability; loss of critical judgment Impairment of perception, memory and comprehension Decreased sensory response; increased reaction time Reduced visual acuity, peripheral vision and glare recovery Sensory-motor incoordination; impaired balance Drowsiness
0.18-0.30	Confusion	Disorientation, mental confusion; dizziness Exaggerated emotional states (fear, rage, grief, etc.) Disturbances of vision (diplopia, etc.) and of perception of color, form, motion, dimensions Increased pain threshold Increased muscular incoordination; staggering gait; slurred speech Apathy, lethargy
0.25-0.40	Stupor	General inertia; approaching loss of motor functions 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 Impairment of circulation and respiration Possible death
0.45 +	Death	Death from respiratory arrest

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Appendix B. Guide to Serum-Alcohol Test Results

SERUM-ALCOHOL CONCENTRATION grams/100 mL.	STAGE OF ALCOHOLIC INFLUENCE	CLINICAL SIGNS/SYMPTOMS
0.01–0.06	Subclinical	No apparent influence Behavior nearly normal by ordinary observation Slight changes detectable by special tests
0.03–0.14	Euphoria	Mild euphoria, sociability, talkativeness Increased self-confidence; decreased inhibitions Diminution of attention, judgment and control Beginning sensory-motor impairment Slowed information processing Loss of efficiency in finer performance tests
0.11–0.29	Excitement	Emotional instability; loss of critical judgment Impairment of perception, memory and comprehension Decreased sensory response; increased reaction time Reduced visual acuity, peripheral vision and glare recovery Sensory-motor incoordination; impaired balance Drowsiness
0.21–0.35	Confusion	Disorientation, mental confusion; dizziness Exaggerated emotional states (fear, rage, sorrow, etc.) Disturbances of vision(diplopia, etc.) and of perception of color, form, motion, dimensions Increased pain threshold Increased muscular incoordination; staggering gait; slurred speech Apathy, lethargy
0.29–0.46	Stupor	General inertia; approaching loss of motor functions Markedly decreased response to stimuli Marked muscular incoordination; inability to stand or walk Vomiting; incontinence of urine and feces Impaired consciousness; sleep or stupor
0.40–0.58	Coma	Complete unconsciousness; coma; anesthesia Depressed or abolished reflexes Subnormal temperature Incontinence of urine and feces Impairment of circulation and respiration Possible death
0.50 +	Death	Death from respiratory arrest

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of Public Health
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RULES AND REGULATIONS CONCERNING
TESTING FOR ALCOHOL AND OTHER DRUGS
APPROVED BY THE COLORADO BOARD OF HEALTH

EFFECTIVE JANUARY 30, 1999

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