

September 1997

Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline



This guideline provides technical and administrative guidance on laboratory procedures related to blood alcohol testing, including specimen collection, methods of analysis, quality assurance, and reporting of results.



NCCLS...

Serving the World's Medical Science Community Through Voluntary Consensus

NCCLS is an international, interdisciplinary, nonprofit, standards-developing and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the healthcare community. It is recognized worldwide for the application of its unique consensus process in the development of standards and guidelines for patient testing and related healthcare issues. NCCLS is based on the principle that consensus is an effective and cost-effective way to improve patient testing and healthcare services.

In addition to developing and promoting the use of voluntary consensus standards and guidelines, NCCLS provides an open and unbiased forum to address critical issues affecting the quality of patient testing and health care.

PUBLICATIONS

An NCCLS document is published as a standard, guideline, or committee report.

Standard A document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A standard may, in addition, contain discretionary elements, which are clearly identified.

Guideline A document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

Report A document that has not been subjected to consensus review and is released by the Board of Directors.

CONSENSUS PROCESS

The NCCLS voluntary consensus process is a protocol establishing formal criteria for:

- The authorization of a project
- The development and open review of documents
- The revision of documents in response to comments by users
- The acceptance of a document as a consensus standard or guideline.

Most NCCLS documents are subject to two levels of consensus—"proposed" and "approved." Depending on the need for field evaluation or data collection, documents may also be made available for review at an intermediate (i.e., "tentative") consensus level.

Proposed An NCCLS consensus document undergoes the first stage of review by the healthcare community as a proposed standard or guideline. The document should receive a wide and thorough technical review, including an overall review of its scope, approach, and utility, and a line-by-line review of its technical and editorial content.

Tentative A tentative standard or guideline is made available for review and comment only when a recommended method has a well-defined need for a field evaluation or when a recommended protocol requires that specific data be collected. It should be reviewed to ensure its utility.

Approved An approved standard or guideline has achieved consensus within the healthcare community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (i.e., that comments on earlier versions have been satisfactorily addressed), and to identify the need for additional consensus documents.

NCCLS standards and guidelines represent a consensus opinion on good practices and reflect the substantial agreement by materially affected, competent, and interested parties obtained by following NCCLS's established consensus procedures. Provisions in NCCLS standards and guidelines may be more or less stringent than applicable regulations. Consequently, conformance to this voluntary consensus document does not relieve the user of responsibility for compliance with applicable regulations.

COMMENTS

The comments of users are essential to the consensus process. Anyone may submit a comment, and all comments are addressed, according to the consensus process, by the NCCLS committee that wrote the document. All comments, including those that result in a change to the document when published at the next consensus level and those that do not result in a change, are responded to by the committee in an appendix to the document. Readers are strongly encouraged to comment in any form and at any time on any NCCLS document. Address comments to the NCCLS Executive Offices, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

VOLUNTEER PARTICIPATION

Healthcare professionals in all specialties are urged to volunteer for participation in NCCLS projects. Please contact the NCCLS Executive Offices for additional information on committee participation.

Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline

Abstract

T/DM6-A, *Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline*, is designed to aid the clinical laboratory in producing timely and accurate blood alcohol results. Its key objective is to address, as comprehensively as possible, recommendations to assure the integrity of the laboratory report on blood alcohol. The document conforms to the objective by addressing specimen collection, methods of analysis, quality assurance, and reporting and significance of results as separate sections. Statutory provisions are included as additional resource information.

The subcommittee recognizes the possible medicolegal impact of blood alcohol testing. The section devoted to the chain-of-custody strives to define the laboratory's responsibility regarding the specimen by outlining specific procedures for the handling and storage of the specimen and subsequent documentation.

[NCCLS. *Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline*. NCCLS Document T/DM6-A (ISBN 1-56238-333-7). NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1997.]

THE NCCLS consensus process, which is the mechanism for moving a document through two or more levels of review by the healthcare community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of NCCLS documents. Current editions are listed in the *NCCLS Catalog*, which is distributed to member organizations, and to nonmembers on request. If your organization is not a member and would like to become one, and to request a copy of the *NCCLS Catalog*, contact the NCCLS Executive Offices. Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: exoffice@nccls.org.

T/DM6-A
ISBN 1-56238-333-7
ISSN 0273-3099

September 1997

Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline

Volume 17 Number 14

Kurt M. Dubowski, Ph.D.
Patricia H. Field, Ph.D.
Walter Frajola, Ph.D.
Vidmantas A. Raisys, Ph.D.
Robert H. Reeder, J.D.
M. Jeffery Shoemaker, Ph.D.
Vana L. Smith, Ph.D.



This publication is protected by copyright. No part of it may be reproduced, stored in a retrieval system, or transmitted in any form or by any means (electronic, mechanical, photocopying, recording, or otherwise) without written permission from NCCLS, except as stated below.

NCCLS hereby grants permission to reproduce limited portions of this publication for use in laboratory procedure manuals at a single site, for interlibrary loan, or for use in educational programs provided that multiple copies of such reproduction shall include the following notice, be distributed without charge, and, in no event, contain more than 20% of the document's text.

Reproduced with permission, from NCCLS publication T/DM6-A, *Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline*. Copies of the current edition may be obtained from NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

Permission to reproduce or otherwise use the text of this document to an extent that exceeds the exemptions granted here or under the Copyright Law must be obtained from NCCLS by written request. To request such permission, address inquiries to the Executive Director, NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

Copyright ©1997. The National Committee for Clinical Laboratory Standards.

Suggested Citation

NCCLS. *Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline*. NCCLS Document T/DM6-A (ISBN 1-56238-333-7). NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1997.

Proposed Guideline

December 1988

Approved Guideline

September 1997

ISBN 1-56238-333-7
ISSN 0273-3099

Committee Membership

Area Committee on Clinical Chemistry and Toxicology

Basil T. Doumas, Ph.D.
Chairholder

Medical College of Wisconsin
Milwaukee, Wisconsin

W. Gregory Miller, Ph.D.
Vice Chairholder

Medical College of Virginia Hospital
Richmond, Virginia

Subcommittee on Blood Alcohol Testing

Kurt M. Dubowski, Ph.D.
Chairholder

The University of Oklahoma
Oklahoma City, Oklahoma

Patricia H. Field, Ph.D.

WI State Laboratory of Hygiene
Madison, Wisconsin

Walter Frajola, Ph.D.

Columbus, Ohio

Vidmantas A. Raisys, Ph.D.

University of Washington
Seattle, Washington

Robert H. Reeder, J.D.

Northwestern University
Evanston, Illinois

M. Jeffery Shoemaker, Ph.D.

Pennsylvania Dept. of Health
Lionville, Pennsylvania

Vana L. Smith, Ph.D.

Abbott Laboratories
Abbott Park, Illinois

Sharon S. Ehrmeyer, Ph.D.
Board Liaison

University of Wisconsin
Madison, Wisconsin

Beth Ann Wise, M.T.(ASCP), M.S.Ed.
Staff Liaison

NCCLS
Wayne, Pennsylvania

Patrice E. Polgar
Editor

NCCLS
Wayne, Pennsylvania

ACTIVE MEMBERSHIP (as of 1 July 1997)**Sustaining Members**

American Association for
Clinical Chemistry
Bayer Corporation
Beckman Instruments, Inc.
Becton Dickinson and Company
Boehringer Mannheim
Diagnostics, Inc.
College of American
Pathologists
Coulter Corporation
Dade International Inc.
Johnson & Johnson Clinical
Diagnostics
Ortho Diagnostic Systems Inc.

Professional Members

American Academy of Family
Physicians
American Association of
Bioanalysts
American Association of Blood
Banks
American Association for
Clinical Chemistry
American Association for
Respiratory Care
American Chemical Society
American Medical Technologists
American Public Health
Association
American Society for Clinical
Laboratory Science
American Society of
Hematology
American Society for
Microbiology
American Society of
Parasitologists, Inc.
American Type Culture
Collection, Inc.
ASQC Food, Drug and Cosmetic
Division
Asociacion Espanola Primera de
Socorros
Association Microbiology Clinici
Italiani-A.M.C.L.I.
Australasian Association of
Clinical Biochemists
Canadian Society of Laboratory
Technologists
Clinical Laboratory Management
Association
College of American
Pathologists
College of Medical Laboratory
Technologists of Ontario

College of Physicians and
Surgeons of Saskatchewan
Commission on Office
Laboratory Accreditation
Institut für Stand. und Dok. im
Med. Lab. (INSTAND)
International Council for
Standardization in
Haematology
International Federation of
Clinical Chemistry
International Society for
Analytical Cytology
Italian Society of Clinical
Biochemistry
Japan Assn. Of Medical
Technologists (Osaka)
Japanese Assn. Of Medical
Technologists (Tokyo)
Japanese Committee for Clinical
Laboratory Standards
Joint Commission on
Accreditation of Healthcare
Organizations
National Academy of Clinical
Biochemistry
National Society for
Histotechnology, Inc.
Ontario Medical Association
Laboratory Proficiency Testing
Program
Sociedade Brasileira de Analises
Clinicas
Sociedad Espanola de Quimica
Clinica
VKCN (The Netherlands)

Government Members

Armed Forces Institute of
Pathology
Association of State and
Territorial Public Health
Laboratory Directors
BC Centre for Disease Control
Centers for Disease Control and
Prevention
China National Centre for the
Clinical Laboratory
Commonwealth of Pennsylvania
Bureau of Laboratories
Department of Veterans Affairs
Deutsches Institut für Normung
(DIN)
FDA Center for Devices and
Radiological Health
FDA Division of Anti-Infective
Drug Products

Government of Malta
Department of Health
Health Care Financing
Administration
INMETRO
Instituto de Salud Publica de
Chile
Istituto Scientifico HS. Raffaele
(Italy)
Iowa State Hygienic Laboratory
Manitoba Health
Massachusetts Department of
Public Health Laboratories
Michigan Department of Public
Health
National Health Laboratory
(Luxembourg)
National Institute of Standards
and Technology
Ohio Department of Health
Oklahoma State Department of
Health
Ontario Ministry of Health
Ordre professionnel des
technologistes médicaux du
Québec
Saskatchewan Health-
Government of Saskatchewan
South African Institute for
Medical Research
Swedish Institute for Infectious
Disease Control

Industry Members

AB Biodisk
Abbott Laboratories
ABC Consulting Group, Ltd.
AccuMed International, Inc.
Aejes
Ammirati Regulatory Consulting
Atlantis Laboratory Systems
Avecor Cardiovascular, Inc.
Bayer Corporation - Elkhart, IN
Bayer Corporation - Middletown,
VA
Bayer Corporation - Tarrytown,
NY
Bayer Corporation - West
Haven, CT
Bayer-Sankyo Co., Ltd.
Beckman Instruments, Inc.
Becton Dickinson and Company
Becton Dickinson Consumer
Products
Becton Dickinson
Immunocytometry Systems
Becton Dickinson Italia S.P.A.

Becton Dickinson Microbiology Systems
 Becton Dickinson VACUTAINER Systems
 Behring Diagnostics Inc.
 Behring Diagnostics Inc. - San Jose, CA
 bioMérieux Vitek, Inc.
 Biometrology Consultants
 Bio-Rad Laboratories, Inc.
 Bio-Reg Associates, Inc.
 Biosite Diagnostics
 Biotest AG
 Boehringer Mannheim Diagnostics, Inc.
 Boehringer Mannheim GmbH
 Bristol-Myers Squibb Company
 Canadian Reference Laboratory Ltd.
 CASCO Standards
 Checkpoint Development, Inc.
 ChemTrak
 Chiron Diagnostics Corporation
 Chiron Diagnostics Corporation - International Operations
 Chiron Diagnostics Corporation - Reagent Systems
 Cholestech
 Clinical Lab Engineering
 COBE Laboratories, Inc.
 Cosmetic Ingredient Review
 Coulter Corporation
 Cytometrics, Inc.
 CYTYC Corporation
 Dade International - Deerfield, IL
 Dade International - Glasgow, DE
 Dade International - Miami, FL
 Dade International - Sacramento, CA
 DAKO A/S
 Diagnostic Products Corporation
 Diametrics Medical, Inc.
 Difco Laboratories, Inc.
 Direct Access Diagnostics
 Eiken Chemical Company, Ltd.
 Enterprise Analysis Corporation
 Donna M. Falcone Consultants
 Fujisawa Pharmaceutical Co. Ltd.
 Gen-Probe
 Glaxo, Inc.
 H&S Consultants
 Health Systems Concepts, Inc.
 Helena Laboratories
 Higman Healthcare
 Hoechst Marion Roussel, Inc.
 Hybritech, Incorporated
 Hycor Biomedical Inc.
 I-STAT Corporation
 Integ, Inc.
 International Biomedical Consultants
 International Remote Imaging Systems (IRIS)
 International Technidyne Corporation
 Johnson & Johnson Clinical Diagnostics
 Johnson & Johnson Health Care Systems, Inc.
 Kimble/Kontes
 Labtest Sistemas Diagnosticos Ltda.
 LifeScan, Inc. (Sub. Ortho Diagnostic Systems Inc.)
 Lilly Research Laboratories
 Luminex Corporation
 Mallinckrodt Sensor Systems
 MBG Industries, Inc.
 Medical Device Consultants, Inc.
 Medical Laboratory Automation Inc.
 MediSense, Inc.
 Merck & Company, Inc.
 Metra Biosystems
 Neometrics, Inc.
 Nissui Pharmaceutical Co., Ltd.
 Norfolk Associates, Inc.
 North American Biologicals, Inc.
 Olympus Corporation
 Optical Sensors, Inc.
 Organon Teknika Corporation
 Orion Diagnostica, Inc.
 Ortho Diagnostic Systems Inc.
 Otsuka America Pharmaceutical, Inc.
 Pfizer Canada, Inc.
 Pfizer Inc
 Pharmacia & Upjohn - Michigan
 Pharmacia & Upjohn - Sweden
 Procter & Gamble Pharmaceuticals, Inc.
 The Product Development Group
 Radiometer America, Inc.
 Radiometer Medical A/S
 Research Inc.
 David G. Rhoads Associates, Inc.
 Rhône-Poulenc Rorer
 Roche Diagnostic Systems (Div. Hoffmann-La Roche Inc.)
 Roche Laboratories (Div. Hoffmann-La Roche Inc.)
 ROSCO Diagnostica
 The R.W. Johnson
 Pharmaceutical Research Institute (Div. Ortho Diagnostic Systems Inc.)
 Sarstedt, Inc.
 Schering Corporation
 Schleicher & Schuell, Inc.
 Second Opinion
 SenDx Medical, Inc.
 Sherwood - Davis & Geck
 Shionogi & Company, Ltd.
 Showa Yakuin Kako Company, Ltd.
 Sienna Biotech
 SmithKline Beecham Corporation
 SmithKline Beecham, S.A.
 SmithKline Diagnostics, Inc. (Sub. Beckman Instruments, Inc.)
 SRL, Inc.
 Streck Laboratories, Inc.
 Sumitomo Metal Bioscience Inc.
 Sysmex Corporation
 TOA Medical Electronics
 Unipath Co (Oxoid Division)
 Vetoquinol S.A.
 Vysis, Inc.
 Wallac Oy
 Warner-Lambert Company
 Wyeth-Ayerst
 Xyletech Systems, Inc.
 Yeongdong Pharmaceutical Corp.
 Zeneca

Trade Associations

Association of Medical Diagnostic Manufacturers
 Health Industry Manufacturers Association
 Japan Association of Clinical Reagents Industries (Tokyo)
 Medical Industry Association of Australia
 National Association of Testing Authorities - Australia

Associate Active Members

Affinity Health System (WI)
 Allegheny University of the Health Sciences (PA)
 Allergy Testing Laboratory (TX)
 Alton Ochsner Medical Foundation (LA)
 American Oncologic Hospital (PA)
 Anzac House (Australia)
 Associated Regional & University Pathologists (UT)
 Baptist Medical Center of Oklahoma
 Baptist Memorial Healthcare System (TX)
 BC Children's Hospital (Canada)
 Bethesda Hospital (OH)
 Beth Israel Medical Center (NY)
 Bristol Regional Medical Center (TN)
 Brooke Army Medical Center (TX)
 Brooks Air Force Base (TX)
 Broward General Medical Center (FL)
 Canterbury Health Laboratories (New Zealand)
 Central Peninsula General Hospital (AK)
 CENTREX Clinical Laboratories (NY)
 Childrens Hospital Los Angeles (CA)
 Children's Hospital Medical Center (Akron, OH)
 Children's Hospital Medical Center (Cincinnati, OH)
 Children's Hospital - New Orleans (LA)
 City Hospital (WV)
 The Cleveland Clinic Foundation (OH)
 Columbia Medical Center (FL)
 Commonwealth of Kentucky CompuNet Clinical Laboratories (OH)
 Consultants Laboratory (WI)
 Dean Medical Center (WI)
 Detroit Health Department (MI)
 Dhahran Health Center (Saudi Arabia)
 Diagnostic Systems Laboratories, Inc. (TX)
 Duke University Medical Center (NC)
 Dunn Memorial Hospital (IN)
 Dwight David Eisenhower Army Medical Center (Ft. Gordon, GA)
 Easton Hospital (PA)
 Ellis Fischel Cancer Center (MO)
 Elyria Memorial Hospital (OH)
 Evanston Hospital (IL)
 Federal Medical Center (MN)
 Fort Leonard Wood Army Community Hospital (MO)
 Frye Regional Medical Center (NC)
 Grady Memorial Hospital (GA)
 Great Smokies Diagnostic Laboratory (NC)
 Hartford Hospital (CT)
 Heritage Hospital (MI)
 Hopital Saint Pierre (Belgium)
 Hunter Area Pathology Service (Australia)
 Incstar Corporation (MN)
 International Health Management Associates, Inc. (IL)
 Jewish Hospital of Cincinnati (OH)
 Kaiser Permanente (CA)
 Kangnam St. Mary's Hospital (Korea)
 Kenora-Rainy River Regional Laboratory Program (Dryden, ON, Canada)
 Klinisches Institute für Medizinische (Austria)
 Laboratoire de Santé Publique du Quebec (Canada)
 Laboratory Corporation of America (NC)
 Lancaster General Hospital (PA)
 Los Angeles County & USC Medical Center (CA)
 Louisiana State University Medical Center
 Maine Medical Center
 Malcolm Grow USAF Medical Center (MD)
 The Medical Center of Ocean County (NJ)
 Melbourne Pathology (Australia)
 Memorial Medical Center (IL)
 Memorial Medical Center (LA)
 Mercy Hospital (MN)
 Methodist Hospital (TX)
 Methodist Hospitals of Memphis (TN)
 Montreal Children's Hospital (Canada)
 Mount Sinai Hospital (NY)
 Mount Sinai Hospital (Toronto, ON, Canada)
 National Genetics Institute (CA)
 Naval Hospital Cherry Point (NC)
 New Britain General Hospital (CT)
 New Hampshire Medical Laboratories
 New Jersey Department of Health
 The New York Blood Center
 New York State Department of Health
 New York State Library
 New York University Medical Center
 North Carolina Laboratory of Public Health
 North Central Bronx Hospital (NY)
 Northwestern Memorial Hospital (IL)
 Olin E. Teague Medical Center (TX)
 Omni Laboratory (MI)
 Our Lady of Lourdes Hospital (NJ)
 Our Lady of the Resurrection Medical Center (IL)
 Palo Alto Medical Foundation (CA)
 PAPP Clinic P.A. (GA)
 Pathogenesis Corp. (WA)
 Pathology Associates Laboratories (CA)
 Permanente Medical Group (CA)
 PLIVA d.d. Research Institute (Croatia)
 Polly Ryon Memorial Hospital (TX)
 Providence Medical Center (WA)
 Puckett Laboratories (MS)
 Queens Hospital Center (NY)
 Quest Diagnostics (MI)
 Ravenswood Hospital Medical Center (IL)
 Reid Hospital & Health Care Services (IN)
 Riverside Clinical Laboratories (VA)
 Royal Brisbane Hospital (Australia)
 St. Boniface General Hospital (Winnipeg, Canada)
 St. Francis Medical Center (CA)
 St. John Regional Hospital (St. John, NB, Canada)
 St. John's Regional Health Center (MO)
 St. Luke's Hospital (PA)
 St. Luke's Regional Medical Center (IA)
 St. Luke's-Roosevelt Hospital Center (NY)
 St. Mary of the Plains Hospital (TX)
 St. Paul Ramsey Medical Center (MN)
 St. Vincent Medical Center (CA)

| | | |
|---|--|---|
| San Francisco General Hospital (CA) | University of Cincinnati Medical Center (OH) | UZ-KUL Medical Center (Belgium) |
| Seoul National University Hospital (Korea) | University of Florida University Hospital (Gent) (Belgium) | VA (Albuquerque) Medical Center (NM) |
| Shanghai Center for the Clinical Laboratory (China) | University Hospital (Linkoping, Sweden) | VA (Denver) Medical Center (CO) |
| Shore Memorial Hospital (NJ) | University Hospital (London, ON, Canada) | VA (Long Beach) Medical Center (CA) |
| SmithKline Beecham Clinical Laboratories (GA) | University Hospital (IN) | VA (Miami) Medical Center (FL) |
| SmithKline Beecham Clinical Laboratories (TX) | University Hospital of Cleveland (OH) | Venice Hospital (FL) |
| South Bend Medical Foundation (IN) | The University Hospitals (OK) | Veterans General Hospital (Republic of China) |
| Southeastern Regional Medical Center (NC) | University of Medicine & Dentistry, NJ University Hospital | Virginia Baptist Hospital |
| Southern California Permanente Medical Group | University of Nebraska Medical Center | Warde Medical Laboratory (MI) |
| SUNY @ Stony Brook (NY) | University of the Ryukyus (Japan) | William Beaumont Hospital (MI) |
| Travis Air Force Base (CA) | The University of Texas Medical Branch | Winn Army Community Hospital (GA) |
| UNC Hospitals (NC) | University of Virginia Medical Center | Wisconsin State Laboratory of Hygiene |
| University of Alberta Hospitals (Canada) | U.S. Army Hospital, Heidelberg | Yonsei University College of Medicine (Korea) |
| University of California, San Francisco | | York Hospital (PA) |
| | | Zale Lipshy University Hospital (TX) |

OFFICERS

A. Samuel Koenig, III, M.D.,
President
Family Medical Care

William F. Koch, Ph.D.,
President Elect
National Institute of Standards and Technology

F. Alan Andersen, Ph.D.,
Secretary
Cosmetic Ingredient Review

Donna M. Meyer, Ph.D.,
Treasurer
Sisters of Charity Health Care System

Charles F. Galanaugh, Past
President
Becton Dickinson and Company (Retired)

John V. Bergen, Ph.D.,
Executive Director

BOARD OF DIRECTORS

Carl A. Burtis, Ph.D.
Oak Ridge National Laboratory

Sharon S. Ehrmeyer, Ph.D.
University of Wisconsin

Elizabeth D. Jacobson, Ph.D.
FDA Center for Devices and Radiological Health

Hartmut Jung, Ph.D.
Boehringer Mannheim GmbH

Tadashi Kawai, M.D., Ph.D.
International Clinical Pathology Center

Kenneth D. McClatchey, M.D., D.D.S.
Loyola University Medical Center

Robert F. Moran, Ph.D.,
FCCM, FAIC
mvi Sciences

David E. Nevalainen, Ph.D.
Abbott Laboratories

Donald M. Powers, Ph.D.
Johnson & Johnson Clinical Diagnostics

Eric J. Sampson, Ph.D.
Centers for Disease Control and Prevention

Marianne C. Watters,
M.T.(ASCP)
Parkland Memorial Hospital

Ann M. Willey, Ph.D.
New York State Department of Health

Contents

| | Page |
|--|------|
| Abstract | I |
| Committee Membership | v |
| Active Membership | vi |
| Foreword | xi |
| | |
| 1 Scope and Requirements | 1 |
| 1.1 Scope of the Problem | 1 |
| 1.2 Medical Requirements | 1 |
| 1.3 Industrial-Medical Requirements | 2 |
| 1.4 Medicolegal Requirements | 2 |
| | |
| 2 Specimen Collection | 3 |
| 2.1 Responsibility for Specimen Collection | 3 |
| 2.2 Types of Specimens | 3 |
| 2.3 Specimen Collection, Handling, and Preservation | 5 |
| 2.4 Specimen Handling | 6 |
| 2.5 Replicate Blood Specimens | 6 |
| | |
| 3 Chain-of-Custody Procedures | 6 |
| 3.1 Purpose | 6 |
| 3.2 Documentation | 7 |
| | |
| 4 Methods of Analysis | 8 |
| 4.1 Gas Chromatography | 8 |
| 4.2 Enzymatic Oxidation with Alcohol Dehydrogenase | 9 |
| | |
| 5 Quality Assurance | 11 |
| 5.1 Calibrators (Standards) | 11 |
| 5.2 Controls | 12 |
| | |
| 6 Reporting and Significance of Results | 12 |
| 6.1 Blood Alcohol Records | 12 |
| 6.2 Conversions | 13 |
| | |
| 7 Terminology and Statutory Provisions | 13 |
| 7.1 Terminology | 13 |
| 7.2 Statutory Provisions | 13 |
| | |
| References | 15 |
| Appendix A: Stages of Acute Alcoholic Influence/Intoxication | 17 |
| Appendix B: Guide to Serum-Alcohol Test Results | 18 |
| Appendix C: Special Specimen Handling Considerations | 19 |
| Summary of Comments and Subcommittee Responses | 20 |
| Related NCCLS Publications | 28 |

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

Dubowski KM © 1994. Reproduced with permission. All rights reserved.

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 μ mol/mL) and sodium fluoride (1.5 mg/mL of blood; 3.6 μ 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 micro-organisms 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 wide-spread 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 handwritten 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 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

*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, <i>via</i> 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

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 backup 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.

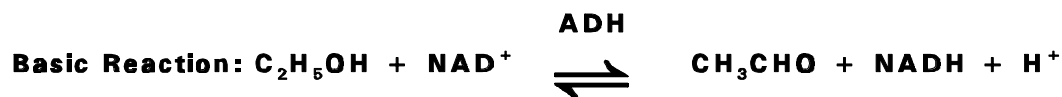
Table 3. Principal Variants of Gas Chromatographic Methods for Blood-Alcohol Analysis

| |
|---|
| 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 |

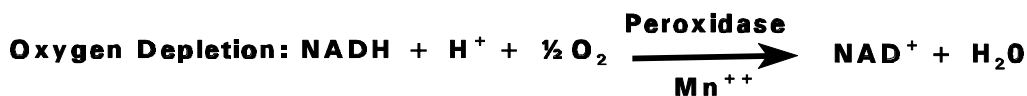
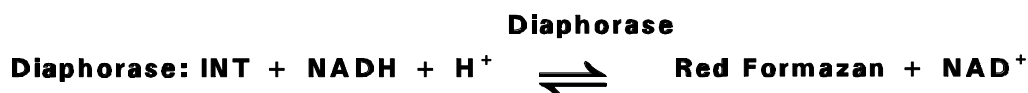
Modified with permission from Dubowski KM. Alcohol determination in the clinical laboratory. *Am J Clin Path.* 1980;74:747-750

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



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 frequently 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: mg/dL X 0.2171 = mmol/L
 mmol/L X 4.61 = 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 figures 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

States 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; RENUMBERED, 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.

References

1. Froede RC. The pathologist's role in the recognition and management of alcoholism. *Am J Clin Pathol.* 74: 718, 1980.
2. Froede RC, Gordon JD. Alcoholism -the second great imitator. An introduction to the problem of alcoholism. *Am J Clin Pathol.* 74: 719-720, 1980.
3. Poklis A. Analytical/Forensic Toxicology. In: *Casarett and Dorell's Toxicology. The Basic Science of Poisons*, 5th ed., ed by C.D. Klaussen. McGraw Hill, New York, 1995, p. 963.
4. Economic issues in alcohol use and abuse. In: *Eighth Special Report to the U.S. Congress on Alcohol and Health from the Secretary of Health and Human Services*, September 1993. NIH Publications No. 94-3699, Rockville, MD: DHHS, National Institute on Alcohol Abuse and Alcoholism, 1994, p. 256.
5. Dubowski KM. Alcohol analysis: Clinical laboratory aspects, Part 1. *Laboratory Management.* 20 (No. 3):43-54, 1982.
6. Dubowski KM, Caplan YH. Alcohol testing in the workplace. In: *Medicolegal Aspects of Alcohol*, 3rd ed. Edited by Garriott JC. Lawyers and Judges Publishing Co., Tucson, Arizona, 1996, pp. 439-475.
7. Dubowski KM. *Manual for Analysis of Ethanol in Biological Liquids.* Report No. DOT-TSC-NHTSA-76-4 (HS 802 208). U.S. Department of Transportation, National Highway Traffic Safety Administration, p. 94, 1977.
8. Kaye S. The collection and handling of blood alcohol specimen. *Am J Clin Pathol.* 74: 743-746, 1980.
9. Brettel HF. *Blutalkohol und Wassergehalt.* Lubeck, Germany, Verlag Max Schmidt-Romhild, 1972, pp. 28-57.
10. Winek CL, Carfagna M. Comparison of plasma, serum, and whole blood ethanol concentrations. *J Analyt Toxic.* 11:267-268, 1987.
11. Rainey PM. Relation between serum and whole-blood ethanol concentrations. *Clin Chem.* 39:2288-2292, 1993.
12. National Committee for Clinical Laboratory Standards. *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture-Second Edition; Approved Standard.* NCCLS document H3-A2. Wayne, Pa.: NCCLS;1984.
13. Dubowski KM, Essary NA. Contamination of blood specimens for alcohol analysis during collection. *Abstracts & Reviews in Alcohol & Driving* 4 (No. 2): 3-7, 1983.
14. Dubowski KM. Alcohol determination in the clinical laboratory. *Am J Clin Pathol.* 74: 747-750, 1980.
15. Blume P, Lakatua DJ. The effect of microbial contamination of the blood sample on the determination of ethanol levels in serum. *Am J Clin Pathol.* 60: 700-702, 1973.
16. Winek CL, Paul LJ. Effect of short-term storage conditions on alcohol concentrations in blood from living human subjects. *Clin Chem.* 29: 1959-1960, 1983.
17. Giannelli PC, Imwinkelried EJ. *Scientific Evidence.* 2nd ed. Charlottesville, VA: The Mitchie Co. 1993; (1):198-214.
18. Moenssens AA, Starrs JE, Henderson CE, and Inbau. *Scientific Evidence in Civil and Criminal Cases.* 4th ed., Westbury, NY: The Foundation Press, Inc., p. 79-80, 1995.
19. Jones AW. Measuring alcohol in blood and breath for forensic purposes - a historical review. *Forensic Science Review* 8(No. 1): 13-43, 1996.
20. Dubowski KM. Ethanol - Type C procedure. In Sunshine, ed. *Methodology for Analytical Toxicology.* Cleveland: CRC Press, Inc. 1975;149-154.
21. Gadsden RH, Terry CS, Thompson BC. Alcohols in biological fluids by gas chromatography (automated head-space method). In: C.S. Frings and W.R. Faulkner, eds. *Selected Methods of Emergency Toxicology.* Washington: AACC Press. 1986;40-43.

22. Shaw RF. Methods for fluid analysis. In J.C. Garriott. ed. *Medicolegal Aspects of Alcohol*, 3rd ed. Tucson, AZ: Lawyers and Judges Publishing Co. 1996; 219–237.
23. Nine JS, et al. Serum-ethanol determination: Comparison of lactate and lactate dehydrogenase interference in three enzymatic assays. *J. Analyt. Toxicol.* 19: 192-196 (1995).
24. Winek CL, Wahba WW. A response to "Serum-ethanol determination. Comparison of lactate and lactate dehydrogenase interference in three enzymatic assays." *J. Analyt. Toxicol.* 20:211-212(1996).
25. *Alcohol and the Impaired Driver*. Chicago, American Medical Association, pp. 143–144, 1970 (Reprinted 1976 by National Safety Council).
26. Uniform Vehicle Code and Model Traffic Ordinance, Revised 1987, Evanston, IL, National Committee on Uniform Traffic Laws and Ordinances, pp. 65–66, 1987.
27. Hardman JG, Limbird LE, Molinoff PB, Ruddon RW. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 9th ed. New York: McGraw-Hill. 1996:386–392
28. Mitchell A. Alcohol-induced impairment of central nervous system function: behavioral skills involved in driving. *J Studies on Alcohol.* 1985; (suppl 10): 106–116, 1985.

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 |

Dubowski KM © 1997. Reproduced with permission. All rights reserved.

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 |

Dubowsk KM © 1991. Reproduced with permission. All rights reserved.

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.

Summary of Comments and Subcommittee Responses

T/DM6-P: *Blood Alcohol Testing in the Clinical Laboratory; Proposed Guideline*

General Comments

1. Further information should be included on the clinical laboratory's obligation pertaining to clinical specimens which become legal issues after the fact. Specimens drawn under clinical orders only are often presented in court cases.
 - **The subcommittee does not believe it feasible to provide details beyond the information in Section 1.4 and interspersed elsewhere, because such matters vary substantially among different jurisdictions and with changing case law.**
2. The document should focus more on the analytical aspects of testing. Gas chromatography (GC) is the method of choice and only 1-1/2 pages discusses GC.
 - **There is ample current information on alcohol analysis readily available in standard sources. These include *J Forensic Sci*, *J. Analyt Toxicol*, *Clin Chem*, *Garriott's Medicolegal Aspects of Alcohol Determination*, 3rd ed., 1996, and many others. This document is not intended to serve as a substitute for these analytical methods resources.**
3. Consider adding a section that lists items that clinical laboratory personnel are often called upon to address during alcohol-related court proceedings.
 - **A checklist of issues which commonly arise in legal proceedings which involve blood-alcohol analysis results appears as Table 1 in Section 1.4.8.**
4. The document indicates plasma, serum, or whole blood may be used. It indicates urine alcohol concentrations are not well correlated with blood alcohol concentrations. It does not mention saliva. I believe the document should say something about the use of saliva (i.e., some literature indicates saliva may be used but this requires further investigation).
 - **While the subcommittee agrees it would be useful information, both breath and saliva testing are beyond the scope of this document. These issues will be referred to the Area Committee on Clinical Chemistry and Toxicology for consideration as the subject of a future NCCLS project.**
5. In our approach to the regulation of alcohol devices, we make a distinction between alcohol analyses obtained for medical purposes and alcohol analyses obtained for legal purposes. We regulate the former but not the latter. Pages 9-10 of the proposed draft indicates this concept is invalid. It states that results obtained for purely medical purposes may ultimately be used for legal purposes. This implies we should regulate all uses and that all uses should have uniform labeling.
 - **Section 1.4 has been reworded to clarify this issue.**
6. The document should be expanded to include concerns of a clinical laboratory as a reference laboratory serving industrial-medical clients (especially in sections 2 and 3) versus off-site specimen collection.
 - **Most matters related to blood alcohol testing in connection with industrial accidents/injuries are covered by the text. Workplace testing for alcohol is a separate subject which is beyond the scope of this document, and one in which blood-alcohol analysis plays a strictly limited role.**

7. We do not agree that a blood alcohol test *must* have legal credibility. Our primary responsibility is to the medical community of our region and to the health and well being of the citizens of our community. Therefore, we provide the blood alcohol determination as a service to the physician in order to determine a disease state without regard to how it may affect the patient's employment and/or his standing with it. We would prefer to maintain a two level system so that a blood alcohol can be measured without regard for the consent by the patient or for the establishment of criminal or civil liability.
 - **A "two-level" system is quite acceptable, as long as there is awareness of potential future legal aspects. Section 1.4 has been reworded to clarify this matter.**
8. I object to the implicit assertion that a clinical laboratory performing blood alcohol testing for medical purposes should institute chain-of-custody procedures. This would greatly increase the cost of providing blood alcohol determinations. This in turn would contribute to the ever increasing spiral of medical costs. Costs would be incurred for all tests although very few results may be subpoenaed. Given the limited effectiveness of increased enforcement in reducing the incidence of driving under the influence, the cost effectiveness of these increased expenditures would be extremely low.
 - **Sections 1.4.4 and 3 have been revised to clarify that chain-of-custody procedures apply chiefly to known medicolegal situations. Moreover, Section 3.2 outlines the choice of omitting chain-of-custody procedures for routine "clinical" situations.**
9. In reviewing the proposed guidelines, I am appreciative that you have taken the time and interest to investigate this area. I believe it is an area that has needed attention in the past. I am concerned, however, that we do not go so far in structuring our testing for the judicial system, that we lose sight of the fact that the physician and his patient are our primary responsibility and purpose.
 - **The subcommittee agrees and the Foreword and Scope of T/DM6-A have been revised to emphasize that the primary responsibility is to the patient and the physician.**
10. Many of the items discussed in this proposed guideline are impractical for a busy clinical laboratory. Two examples of this are the requirement for controlled access to all specimens (Section 3.2) and the suggestion that records should be maintained for each case in an individual file (Section 6.1). These are not practical for a clinical laboratory serving an emergency room for instance, and will actually slow down the speed of testing.
 - **The subcommittee agrees and Section 6.1 has been revised to include the use of computers in record-keeping.**
11. To be practical, it is necessary to distinguish between those specimens drawn to make a clinical diagnosis, and those drawn as evidence in a legal proceeding. The two situations are not compatible; a legal process is deliberately slow, while a clinical diagnosis on an emergency patient is deliberately fast. It is unacceptable to slow down the process of arriving at a clinical diagnosis on the basis of laboratory data based on the possibility that the results might be used in some legal process in the future. If such a legal process is anticipated, then two specimens can be drawn at the same time, one to be sent quickly for a medical diagnosis, and the other sent through the slow, deliberate process to be used as evidence. What I think should be addressed by this proposed guideline are criteria for what constitutes reasonable sample handling for the medical process.
 - **The subcommittee agrees that drawing replicate specimens may streamline the process. See Section 2.5.**

12. Nowhere in this proposed guideline is the problem of calculating blood alcohol concentration at some previous time based on a specimen collected later discussed. I'm sure that the authors did this deliberately, feeling that such calculations are really the providence of an expert witness. If this is the case, they should state so explicitly.
- **The subcommittee considers the issue of extrapolation beyond the scope of the document. Section 1.4.7 has been revised to so indicate.**

Section 1.0

13. In Section 1.2, page 186, the last sentence refers only to clinical chemistry tests in a laboratory evaluation of a suspected alcohol-related patient condition. A laboratory workup also might include hematologic and histopathologic tests.
- **The document has been revised to include all relevant laboratory tests.**
14. I concur that gas chromatography (GC) methods are more specific and are the methods of choice. However, there is no discussion of whether *confirmation* is appropriate, particularly if methods other than GC are used. Some discussion of the need for confirmation is appropriate in light of the statement in Section 1.4 that medical purposes cannot be separated from legal purposes.
- **Section 1.4.6 has been revised to address this comment.**
15. I would like to take issue with the following statement: "The concept of obtaining a blood specimen for medical purposes only is invalid because in most legal jurisdictions the laboratory results can be subpoenaed in some circumstances." Obtaining a blood alcohol with the *intent* that it may later be used in a criminal proceeding without obtaining a patient's consent for that use would in many states be a violation not only of the patient's legal rights, but also of the physician-patient privilege. The setting up of chain-of-custody procedures for alcohol determinations in a laboratory which does not do forensic testing could be considered prima facie evidence that there was intent to use this result for legal purposes.
- **The relevant language in Section 1.4 has been revised to clarify the issue. The subcommittee, however, does not agree with the commentor's last two sentences.**
16. I disagree with statements in Section 1.4. We run specimens for medical purposes only.
- **The relevant language in Section 1.4 and elsewhere has been revised to clarify the issue.**

Section 2.0

17. In Section 2.2, page 190, the first paragraph states that plasma and serum are more appropriate specimens than whole blood for analysis, but then fails to state why whole blood is generally used.
- **Section 2.2 has been reworded to clarify this matter.**
18. Some states have passed an "implied consent law." What do you think the laboratory's responsibility should be as far as obtaining consent from the patient. If another department (such as the Emergency Room) takes care of having the consent forms signed, what do you feel to be the laboratory's responsibility for ensuring that the forms have actually been signed prior to specimen collection?
- **Because requirements vary significantly from state to state, it is necessary to obtain and follow your state's regulation regarding informed consent. Recent case law has uniformly held that separate and additional hospital (or laboratory) consent may not be demanded from a person who has consented to an implied-consent blood-alcohol test.**

19. Section 2.2, page 191, paragraph 3 recommends that laboratories not convert serum or plasma values for alcohol to the whole blood equivalent, but states that courts should retain an expert to make this conversion. At least a reference should be given explaining the problems with making such conversions that would require an expert.
 - **A reference has been added as suggested.**
20. If serum/plasma as a specimen is allowed there should also be help in interpretation.
 - **Appendix B has been added to assist in the interpretation of serum-alcohol concentrations.**
21. Although povidone-iodine may be acceptable for cleansing the venipuncture site when only a blood alcohol is to be obtained, it has been reported to interfere with other clinical assays and should not be used when a specimen is being obtained for multiple tests which include a blood alcohol level.
 - **The recommendations in this document focus on specimen collection for alcohol testing. For further information consult NCCLS document H3: *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture*.**
22. Sending samples out through the mail seems to be a procedure that would lead to legal problems.
 - **If accompanied by full identification and laboratory test report and mailed via registered mail or certified mail, no problems should be encountered.**
23. Section 2.3.4, page 195, should the collection of two specimens be considered instead of one when the specimen is almost certain to be involved in a medical legal question?
 - **There is nothing to preclude taking two specimens if it is not clinically contraindicated and it meets local legal rules. See response to Comment 11 above. A new Section 2.5 also discusses this matter.**
24. In Section 2.3.4, I recommend that a specific time period be used in "If the specimen is to be analyzed for alcohol content within a period of a few hours." Also use specific time period in the second paragraph.
 - **The document has been revised to include a specific time period (i.e., within four hours).**
25. In Section 2.3.4, the statement "If the analysis will be delayed. . .sufficient sodium fluoride to produce a minimum concentration of 10 mg/mL" is inconsistent with a statement on page 194 which indicates a 1.5 mg/mL NaF for short-term storage at 5 degrees C. Also a specific time period should be specified in place of "short-term."
 - **See response to Comment 24 above. The statements are both correct. The higher NaF concentration is needed if the specimen is not refrigerated or is transported.**
26. Please define "extended periods of time" (page 194, second paragraph.)
 - **Section 2.3.4 has been reworded.**
27. We don't usually identify the venipuncture site as indicated in Section 2.4. Is it necessary?
 - **Yes, because of i.v. sites. Blood collection, preparation of the puncture site, and related issues are frequently raised in relation to blood-alcohol test results.**

Section 3.0

28. We have a policy that a single technologist or technician draws the specimen, handles it exclusively, and performs the analysis for ethanol. We have done this mainly to avoid having more than one employee subpoenaed to testify in court. Therefore, we do not have a chain of custody form, tamper-evident seals, or locked storage for our alcohols performed in house. Do you see a problem with this?
- **The subcommittee considers that this system, with proper documentation by the technologist, can obviate the need for more complex procedures.**

Section 4.0

29. The discussion of each analytical method should ideally address expected CVs.
- **The subcommittee considers this beyond the scope of the document. Expected CVs are specific to the laboratory and methodology; therefore, each laboratory should establish its own.**
30. Three methods listed in the order of preference are stated as appropriate for clinical laboratories. They are: gas chromatography (GC), enzymatic oxidation with alcohol dehydrogenase, and osmometry. There is no mention of alcohol test strips employing alcohol dehydrogenase. I believe the draft could make some kind of statement about such devices (i.e., they have been reported in the literature but require further evaluation).
- **Osmometry has been eliminated from the document. The other subject is beyond the scope of the document.**
31. It should be stated that osmometry, while helpful in clinical situations, is not suitable for legal proceedings.
- **Section 4.3 has been deleted in T/DM6-A.**
32. In Section 4.2, you also say, "All ADH-based enzymatic oxidation methods are subject to potential interference by isopropanol and higher alcohols to varying extents..." Published studies indicate that the "ADH" enzyme currently in use is very specific for ethanol and that isopropanol and similar organic substances (methanol, acetone, ethylene glycol) do not affect results.
- **High concentrations of isopropanol and higher alcohols do affect these results. The extent of the potential interference is a function of the individual test kit. The section has been reworded to clarify that commonly-encountered concentrations of methanol and isopropanol do not significantly interfere.**
33. In Section 4.3, page 206, an example of an osmolality calculation might be useful to include in this section. The reference cited in the text might not be readily available in many laboratories, especially in those laboratories not performing toxicology measurements on a regular basis.
- **This section has been deleted in T/DM6-A.**
34. The enzymatic method and method involving freezing point depression ought to be discouraged since they are susceptible to interference from other alcohols.
- **Freezing point osmometry has been deleted. Enzymatic methods are the most widely used test methods for clinical applications. It is impractical to discourage their use at this time.**
35. I would like to see a more detailed discussion of enzymatic methods, i.e., greater distinction between alcohol dehydrogenase (ADH) and alcohol oxidase (AO), especially since most hospitals use ADH.

- **Because of the length of such additional information, and changing commercial kits, it is not feasible to incorporate that information into the document.**
36. Maximum information on analytical interferences for all generic and/or specific methods considered acceptable should be included.
- **The subcommittee considers this beyond the scope of the document.**
37. The listing of methods in Table 1, Summary of Methods for Blood Alcohol Analysis is incomplete. Two methods used by some states are not listed; namely, Chemical Oxidation Following Distillation to Separate Alcohol from Blood, and Chemical Oxidation Following Diffusion to Separate Alcohol from Blood. It may be that these methods were omitted because of the limitation set forth in Paragraph 4.0 of the document that these are methods "appropriate for use in clinical laboratories." Nevertheless, the focus of the document is the forensic application of the blood alcohol test, especially as related to drunk driver arrests. Therefore, it appears reasonable to me that the document should strive for completeness by listing and describing these two versions of the chemical, oxidation-reductions tests for alcohol in blood.
- **Chemical oxidation has become nearly extinct, and the subcommittee considers a discussion of it unnecessary.**
38. The methods of analysis section was short and did not provide as much information concerning analysis and interferences that are possible in the analysis.
- **The subcommittee considers this beyond the scope of the document. For further information consult *Medicolegal Aspects of Alcohol*. J. Garritt (Ed.), 3rd ed., Tucson, AZ, Lawyers & Judges Publishing Co., 1996.**
39. Are you sure that sodium fluoride doesn't inhibit enzymatic methods?
- **If used in the concentrations given, sodium fluoride doesn't inhibit enzymatic methods for ethanol analysis, as evidenced by the CAP Survey results for Surveys AL1 and AL2.**
40. Would you comment on the interference by hemolysis in the ACA method, as hemolysis is a problem which commonly results from the use of potassium oxalate/NaF in the blood collection tube?
- **Interference in spectrophotometric methods for chemical analysis using enzymatic oxidation can be avoided by preparing a 1:3 or 1:4 protein-free filtrate and analyzing it. For details, consult Gadsden RH and Taylor EH. Ethanol in biological fluids by enzymatic analysis. In: *Selected Methods of Emergency Toxicology*. Washington DC, AACC Press, 1986, pp. 63–65.**
41. Please add references for interference by isopropanol, higher alcohols (these should be named), and methanol.
- **Such information should be obtained from the manufacturer of a given reagent kit, such as package inserts and analysis manuals.**

Section 5.0

42. I recommend that Section 5.0, Quality Assurance, begin with a new section 5.1 entitled "Blanks." The value and usefulness of blank samples in quantitative procedures is well established. Their particular value and usefulness in the forensic application of blood alcohol tests is clear.
- **The use of blank specimens is required but their incorporation in the test procedure is method dependent.**

43. Under Section 5.1 "Calibrators (Standards)," the density of ethanol should be stated. This would assist people who are preparing standards on a weight basis using volumetric equipment.
- **That information is readily available in standard reference manuals, e.g., The Merck Index, which should be available in all laboratories.**
44. The requirement to run a calibrator with every analysis and every batch, discussed in Section 5.1, is unreasonable and excessive for many analytical systems. The requirement should specify that this be done for GC methods or on batch or random access analyzers that require standards with each analytical run for any test.
- **Sections 5 and 5.1 have been rewritten to clarify this point. However, it is good practice to run at least one positive and one negative control with each patient sample for a determination that has such high probability of legal consequences as alcohol; documentation is important.**
45. Under Section 5.2, "Controls," the frequency that controls should be run does not correspond to the requirements for some instruments. This enzymatic method is in frequent use in the clinical laboratories. Generally controls are run on this instrument once a day. If the authors feel that this is insufficient for legal proceedings, then this problem should be addressed specifically. This is true of the standards also.
- **See response to Comment 44.**
46. I question the need to initiate every alcohol test with calibrators.
- **See response to Comment 44.**
47. I recommend the addition of two concepts to Section 5.2, "Controls." First, it should be stated that when a laboratory is setting the mean and standard deviation for its controls, the 10 or 20 replicate analyses should be done at the rate of no more than 2 per day. Secondly, the goal of a quality control program would be better stated if, at the end of Section 5.2, there were language to the effect that: "Whenever analysis of controls is outside the acceptable limits, the method shall be regarded to be in error, and the laboratory must take remedial action to investigate and correct the source of error. Until such time that the error has been corrected, as shown by return of analysis of controls to values within the acceptable limits, no samples will be analyzed for blood alcohol."
- **A brief comment has been added to Section 5.2 to confirm the need for action when out-of-control results occur; and for assignment of control values and limits, reference has been made to NCCLS document C24 to clarify these matters.**
48. You made reference to control in this guideline, but we have not been able to locate a whole blood control. Can you specify the type of controls you make reference to?
- **There are commercial preparations listed in laboratory supply catalogs and available through manufacturers of control materials.**

Section 6.0

49. We have a notebook which lists patients by name and number as well as their ethanol concentration and the value of the control that was run. It also includes the date, time, and initials of the person performing the test. It is a chronological file and we have no alphabetical or numerical cross-reference. It is stored on a shelf in our chemistry lab and it is not locked. Are you suggesting a computer cross-reference system? Do you feel our present filing system is adequate? You suggested a ten year storage time. The statute of limitations has been shortened, is this still your recommended time?

- **Any system is subject to challenge, but one can minimize the likelihood of successful challenge. The notebook mentioned in this comment should be kept under lock and key when not in actual use in the laboratory.**

Section 7.0

50. On page 211 Section 7.1.2, referring to Blood Alcohol Concentration (BAC) with respect to rounding off from 3 digits to 2 digits, you state 139 mg/dl, 0.139% (g/dl) would round off to 0.13%. Are you saying that the third digit is always dropped rather than traditional rounding off. For example, would 0.099 round off to 0.09 or to 0.10?
- **Yes, the universal practice is to *truncate*, i.e., drop the third decimal entirely, not round upward. This section has been revised to clarify when and how you round to a two-digit value.**
51. I would like to see a reference for the statement "some individuals are impaired at less than 0.10% w/v; others are not *measurably* impaired at much higher concentrations." I am not aware of any studies which show that there are individuals whose performance is the same at blood alcohol levels of 0.0 and 0.1% when measured quantitatively (e.g., reaction time). If there are no such studies, perhaps it would be better to state that "others are not *obviously* impaired at much higher concentrations" or that some individuals may appear to be unimpaired at much higher concentrations.
- **The document has been changed as suggested (see Section 7.2).**
52. I think the expression of concentration as percent should be eliminated. If % (w/v) = g/dl, then let's say so. Then there would be no assumption as to whether we mean % (w/v) or % (w/w). This sometimes is a major problem in court.
- **The percent weight/volume notation is ingrained in many regional laws and state regulations, and some federal laws. The subcommittee cannot affect these practices.**

Related NCCLS Publications[†]

- C24-A Internal Quality Control Testing: Principles and Definitions; Approved Guideline (1991).** Discusses the purpose of internal quality control; defines various analytical intervals, such as "analytical run"; and addresses the use of quality control material and control data, including the use of data in quality assurance and interpretation.
- EP10-T2 Preliminary Evaluation of Quantitative Clinical Laboratory Methods - Second Edition; Tentative Guideline (1993).** Discusses experimental design and data analysis for preliminary evaluation of the performance
- GP2-A3 Clinical Laboratory Technical Procedure Manuals—Third Edition; Approved Guideline (1996).** Offers guidelines that address the design, preparation, maintenance, and use of technical procedure manuals in the clinical laboratory.
- H3-A3 Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture—Third Edition; Approved Standard (1991).** Discusses methods for the collection of blood specimens by venipuncture and an appropriate training program aimed at increasing analyte integrity and minimizing laboratory error. Includes a 24-step protocol for specimen collection, recommendations for "order of draw," and considerations for performing venipuncture on children.
- H5-A3 Procedures for the Handling and Transport of Diagnostic Specimens and Etiologic Agents-Third Edition; Approved Standard (1994).** *American National Standard.* Gives proper packaging, handling, and transport requirements for medical specimens. Includes federal regulations.
- H18-A Procedures for the Handling and Processing of Blood Specimens; Approved Guideline (1990).** Addresses the multiple factors associated with handling and processing specimens, factors that can introduce imprecision or systematic bias into test results.
- H31-P Collection Containers for Specimens for Toxicological Analysis; Proposed Guideline (1986).** Discusses recommended toxicology/drug monitoring requirements for blood collection containers.
- M29-T2 Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue—Second Edition; Tentative Guideline (1991).** Guidance on the risk of transmission of hepatitis B virus and human immunodeficiency viruses in the laboratory; specific precautions for preventing transmission of bloodborne infection during clinical and anatomical laboratory procedures.

[†] Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.