C40-A
Vol. 21 No. 9

Replaces C40-P
Vol. 18 No. 4

Analytical Procedures for the Determination of Lead in Blood and Urine; Approved Guideline

This document provides guidelines for the measurement of lead in blood and urine, including specimen collection, measurement by graphite furnace atomic absorption spectrometry (GFAAS) and anodic stripping voltammetry (ASV), quality assurance, and quality control.

A guideline for global application developed through the NCCLS consensus process.

NCCLS

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.

Analytical Procedures for the Determination of Lead in Blood and Urine; Approved Guideline

Abstract

Analytical Procedures for the Determination of Lead in Blood and Urine; Approved Guideline (NCCLS document C40-A) is intended for use by the clinical laboratory testing community involved in the collection and measurement of lead in blood and urine. The guideline addresses the clinical significance of lead measurements, specimen collection, lead determination by graphite furnace atomic absorption spectrometry and anodic stripping voltammetry, reference materials, quality control procedures, and laboratory policy.

NCCLS. *Analytical Procedures for the Determination of Lead in Blood and Urine; Approved Guideline*. NCCLS document C40-A (ISBN 1-56238-437-6). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2001.

C40-A
ISBN 1-56238-437-6
ISSN 0273-3099

Analytical Procedures for the Determination of Lead in Blood and Urine; Approved Guideline

Volume 21 Number 9

Patrick J. Parsons, Ph.D., C.Chem., FRSC, Chairholder
J. Julian Chisolm, Jr., M.D.
H. Trevor Delves, Ph.D., C.Chem., FRSC
Reginald Griffin, Ph.D.
Elaine W. Gunter, M.T.(ASCP)
Walter Slavin
Noel V. Stanton, M.S.
Robert Vocke, Ph.D.

Suggested Citation

(NCCLS. *Analytical Procedures for the Determination of Lead in Blood and Urine; Approved Guideline.* NCCLS document C40-A [ISBN 1-56238-437-6]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2001.)

Proposed Guideline
April 1998

Approved Guideline
June 2001

ISBN 1-56238-437-6
ISSN 0273-3099

Committee Membership

Area Committee on Clinical Chemistry and Toxicology

W. Gregory Miller, Ph.D. **Chairholder**	**Virginia Commonwealth University** **Richmond, Virginia**
Gary L. Myers, Ph.D. **Vice-Chairholder**	**Centers for Disease Control and Prevention** **Atlanta, Georgia**

Subcommittee on Determination of Lead

Patrick J. Parsons, Ph.D., C.Chem, FRSC **Chairholder**	**New York State Department of Health** **Albany, New York**
J. Julian Chisolm, Jr., M.D.	Kennedy Krieger Institute Baltimore, Maryland
H. Trevor Delves, Ph.D., C.Chem., FRSC	Southampton General Hospital Southampton, United Kingdom
Reginald Griffin, Ph.D.	ESA Laboratories Chelmsford, Massachusetts
Elaine W. Gunter, M.T.(ASCP)	Centers for Disease Control and Prevention Atlanta, Georgia
Walter Slavin	Bonaire Technologies Ridgefield, Connecticut
Noel V. Stanton, M.S.	WI State Lab of Hygiene Madison, Wisconsin
Robert Vocke, Ph.D.	National Institute of Standards and Technology Gaithersburg, Maryland

Advisors

Derek P. Baker, Ph.D.	Utak Laboratories Agoura Hills, California
Glen Carnrick	Perin-Elmer Corporation Wilton, Connecticut
V. Michelle Chenault, Ph.D., M.T.(ASCP)	Food and Drug Administration Rockville, Maryland
John W. Erickson, Jr.	Bio-Rad Laboratories Anaheim, California
Robert Jones, Ph.D.	Centers for Disease Control and Prevention Atlanta, Georgia

Advisors (Continued)

Fred Y. Leung, Ph.D.	University Hospital, Ontario
Gillian Lockitch, M.D., FRCPC	Children's & Women's Health Center Vanouver, British Columbia
John D. Osterloh, M.D.	San Francisco General Hospital San Francisco, California
Daniel Paschal, Ph.D.	Centers for Disease Control and Prevention Atlanta, Georgia
Amadeo J. Pesce, Ph.D.	University Hospital Cincinnati, Ohio
Petrie Rainey, M.D., Ph.D.	Yale School of Medicine New Haven, Connecticut
Sandy Roda	University of Cincinnati Cincinnati, Ohio
Kenneth L. Seace	Buck Scientific East Norwalk, Connecticut
Douglas E. Shrader, Ph.D.	Varian Institute Wood Dale, Illinois
John Sotera	Thermo Jarrel Ash Corporation Franklin, Massachusetts
Dr. Andrew Taylor	University of Guildford Surrey, England
Beth Ann Wise, M.T.(ASCP), M.S.Ed. *Staff Liaison*	NCCLS Wayne, Pennsylvania
Patrice E. Polgar *Editor*	NCCLS Wayne, Pennsylvania
Donna M. Wilhelm *Assistant Editor*	NCCLS Wayne, Pennsylvania

Active Membership
(as of 1 April 2001)

Sustaining Members

Abbott Laboratories
American Association for Clinical Chemistry
Bayer Corporation
Beckman Coulter, Inc.
BD and Company
bioMérieux, Inc.
College of American Pathologists
Dade Behring Inc.
GlaxoSmithKline
Nippon Becton Dickinson Co., Ltd.
Ortho-Clinical Diagnostics, Inc.
Pfizer Inc
Roche Diagnostics, Inc.

Professional Members

AISAR-Associazione Italiana per lo Studio degli
American Academy of Family Physicians
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.
Asociacion de Laboratorios de Alta Complejidad
Asociación Española Primera de Socorros (Uruguay)
Asociacion Mexicana de Bioquimica Clinica A.C.
Assn. of Public Health Laboratories
Assoc. Micro. Clinici Italiani-A.M.C.L.I.
Australasian Association of Clinical Biochemists
British Society for Antimicrobial Chemotherapy
CADIME-Camara De Instituciones De Diagnostico Medico
Canadian Society for Medical Laboratory Science—Société Canadienne de Science de Laboratoire Médical
Canadian Society of Clinical Chemists
Clinical Laboratory Management Association
COLA
College of American Pathologists
College of Medical Laboratory Technologists of Ontario
College of Physicians and Surgeons of Saskatchewan
Fundación Bioquímica Argentina
International Association of Medical Laboratory Technologists
International Council for Standardization in Haematology
International Federation of Clinical Chemistry
Italian Society of Clinical Biochemistry
Japan Society of Clinical Chemistry
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 Quality Management Program-Laboratory Service
RCPA Quality Assurance Programs PTY Limited
Sociedade Brasileira de Analises Clinicas
Sociedade Brasileira de Patologia Clinica
Sociedad Espanola de Bioquimica Clinica y Patologia Molecular

Government Members

Association of Public Health Laboratories
Armed Forces Institute of Pathology
BC Centre for Disease Control
Centers for Disease Control and Prevention
Chinese Committee for Clinical Laboratory Standards
Commonwealth of Pennsylvania Bureau of Laboratories
Department of Veterans Affairs
Deutsches Institut für Normung (DIN)
FDA Center for Devices and Radiological Health
FDA Center for Veterinary Medicine
FDA Division of Anti-Infective Drug Products
Health Care Financing Administration/CLIA Program
Health Care Financing Administration
Iowa State Hygienic Laboratory
Massachusetts Department of Public Health Laboratories
National Association of Testing Authorities – Australia
National Center of Infectious and Parasitic Diseases (Bulgaria)
National Institute of Standards and Technology
Ohio Department of Health
Ontario Ministry of Health
Saskatchewan Health-Provincial Laboratory
Scientific Institute of Public Health; Belgium Ministry of Social Affairs, Public Health and the Environment
South African Institute for Medical Research
Swedish Institute for Infectious Disease Control
Thailand Department of Medical Sciences

Industry Members

AB Biodisk
Abbott Laboratories
Abbott Laboratories, MediSense Products
Accumetrics, Inc.
Agilent Technologies, Inc.
Ammirati Regulatory Consulting
Asséssor
AstraZeneca
Aventis
Avocet Medical, Inc.
Bayer Corporation – Elkhart, IN

Bayer Corporation – Tarrytown, NY
Bayer Corporation – West Haven, CT
Bayer Medical Ltd.
BD
BD Biosciences – San Jose, CA
BD Consumer Products
BD Diagnostic Systems
BD Italia S.P.A.
BD VACUTAINER Systems
Beckman Coulter, Inc.
Beckman Coulter, Inc. Primary Care Diagnostics
Beckman Coulter K.K. (Japan)
Bio-Development SRL
Bio-Inova Life Sciences International
Bio-Inova Life Sciences North America
BioMedia Laboratories Sdn Bhd
bioMérieux, Inc.
Biometrology Consultants
Bio-Rad Laboratories, Inc.
Bio-Rad Laboratories, Inc. - France
Biotest AG
Bristol-Myers Squibb Company
Canadian External Quality Assessment Laboratory
Capital Management Consulting, Inc.
Checkpoint Development Inc.
Clinical Design Group Inc.
Clinical Laboratory Improvement Consultants
COBE Laboratories, Inc.
Community Medical Center (NJ)
Control Lab (Brazil)
Copan Diagnostics Inc.
Cosmetic Ingredient Review
Cubist Pharmaceuticals
Cytometrics, Inc.
Dade Behring Inc. - Deerfield, IL
Dade Behring Inc. - Glasgow, DE
Dade Behring Inc. - Marburg, Germany
Dade Behring Inc. - Sacramento, CA
Dade Behring Inc. - San Jose, CA
DAKO A/S
Diagnostic Products Corporation
Eiken Chemical Company, Ltd.
Enterprise Analysis Corporation
Fort Dodge Animal Health
General Hospital Vienna (Austria)
Gen-Probe
GlaxoSmithKline
Greiner Bio-One Inc.
Health Systems Concepts, Inc.
Helena Laboratories
Home Diagnostics, Inc.
I-STAT Corporation
International Technidyne Corporation
Kendall Sherwood-Davis & Geck
LAB-Interlink, Inc.
Labtest Diagnostica S.A.
LifeScan, Inc. (a Johnson & Johnson Company)
Lilly Research Laboratories
Medical Device Consultants, Inc.
Medtronic, Inc.
Merck & Company, Inc.
mvi Sciences (MA)
Nabi
Neometrics is.
Nichols Institute Diagnostics (Div. of Quest Diagnostics, Inc.)
Nissui Pharmaceutical Co., Ltd.
Nippon Becton Dickinson Co., Ltd.
Norfolk Associates, Inc.
Organon Teknika Corporation
Ortho-Clinical Diagnostics, Inc. (Raritan, NJ)
Ortho-Clinical Diagnostics, Inc. (Rochester, NY)
Oxoid Inc.
Pfizer Inc
Pharmacia Corporation
Premier Inc.
Procter & Gamble Pharmaceuticals, Inc.
The Product Development Group
Quintiles, Inc.
Radiometer America, Inc.
Radiometer Medical A/S
David G. Rhoads Associates, Inc.
Roche Diagnostics GmbH
Roche Diagnostics, Inc.
Roche Laboratories (Div. Hoffmann-La Roche Inc.)
The R.W. Johnson Pharmaceutical Research Institute
Sarstedt, Inc.
SARL Laboratoire Carron (France)
Schering Corporation
Schleicher & Schuell, Inc.
Second Opinion
Showa Yakuhin Kako Company, Ltd.
Streck Laboratories, Inc.
SurroMed, Inc.
Sysmex Corporation (Japan)
Sysmex Corporation (Long Grove, IL)
The Toledo Hospital (OH)
Trek Diagnostic Systems, Inc.
Vetoquinol S.A.
Visible Genetics, Inc.
Vysis, Inc.
Wallac Oy
Wyeth-Ayerst
Xyletech Systems, Inc.
YD Consultant
Yeongdong Pharmaceutical Corporation

Trade Associations

AdvaMed
Association of Medical Diagnostic Manufacturers
Japan Association Clinical Reagents Ind. (Tokyo, Japan)
Medical Industry Association of Australia

Associate Active Members

20th Medical Group (SC)
67th CSH Wuerzburg, GE (NY)
121st General Hospital (CA)
Academisch Ziekenhuis-VUB (Belgium)
Acadiana Medical Laboratories, LTD (LA)
Adena Regional Medical Center (OH)
Advocate Laboratories (IL)
The Aga Khan Hospital & Medical College, Karachi (Pakistan)
Akershus Central Hospital and AFA (Norway)
Albany Medical Center Hospital (NY)
Albemarle Hospital (NC)
Allegheny General Hospital (PA)
Allegheny University of the Health Sciences (PA)
Allina Laboratories (MN)
Alton Ochsner Medical Foundation (LA)
American Medical Laboratories (VA)
Arkansas Department of Health
ARUP at University Hospital (UT)
Armed Forces Research Institute of Medical Science (APO, AP)
Associated Regional & University Pathologists (UT)
Aurora Consolidated Laboratories (WI)
Bay Medical Center (MI)
Baystate Medical Center (MA)
Bbaguas Duzen Laboratories (Turkey)
Bo Ali Hospital (Iran)
Bonnyville Health Center (Alberta, Canada)

Boulder Community Hospital (CO)
Brantford General Hospital (Ontario, Canada)
Brazileiro De Promocao (Brazil)
Brookdale Hospital Medical Center (NY)
Brooke Army Medical Center (TX)
Brooks Air Force Base (TX)
Broward General Medical Center (FL)
Calgary Laboratory Services
Carilion Consolidated Laboratory (VA)
Cathay General Hospital (Taiwan)
CB Healthcare Complex (Sydney, NS, Canada)
Central Kansas Medical Center
Central Texas Veterans Health Care System
Centro Diagnostico Italiano (Milano, Italy)
Champlain Valley Physicians Hospital (NY)
Chang Gung Memorial Hospital (Taiwan)
Children's Hospital (LA)
Children's Hospital (NE)
Children's Hospital & Clinics (MN)
Children's Hospital King's Daughters (VA)
Children's Hospital Medical Center (Akron, OH)
Children's Hospital of Philadelphia (PA)
Clarian Health–Methodist Hospital (IN)
Clendo Lab (Puerto Rico)
CLSI Laboratories (PA)
Columbus County Hospital (NC)
Commonwealth of Kentucky
CompuNet Clinical Laboratories (OH)
Covance Central Laboratory Services (IN)
Danish Veterinary Laboratory (Copenhagen, Denmark)
Danville Regional Medical Center (VA)
Deaconess Hospital (MO)
Delaware Public Health Laboratory
Department of Health & Community Services (New Brunswick, Canada)
Detroit Health Department (MI)
Diagnostic Laboratory Services, Inc. (HI)
Duke University Medical Center (NC)
Durham Regional Hospital (NC)
Dynacare Laboratories - Eastern Region (Ottawa, ON, Canada)
Dynacare Memorial Hermann Laboratory Services (TX)
E.A. Conway Medical Center (LA)
Eastern Maine Medical Center
East Side Clinical Laboratory (RI)
Elyria Memorial Hospital (OH)
Emory University Hospital (GA)
Esoterix Center for Infectious Disease (TX)
Fairfax Hospital (VA)
Fairview-University Medical Center (MN)
Foothills Hospital (Calgary, AB, Canada)
Fort St. John General Hospital (Fort St. John, BC, Canada)
Fox Chase Cancer Center (PA)
Franklin Square Hospital Center (MD)
Fresenius Medical Care/Spectra East (NJ)
Fresno Community Hospital and Medical Center
Gambro Healthcare Laboratory (FL)
GDS Technology, Inc (IN)
Geisinger Medical Center (PA)
Grady Memorial Hospital (GA)
Guthrie Clinic Laboratories (PA)
Harris Methodist Erath County (TX)
Harris Methodist Fort Worth (TX)
Harris Methodist Northwest (TX)
Hartford Hospital (CT)
Headwaters Health Authority (Alberta, Canada)
Health Network Lab (PA)
Health Sciences Centre (Winnipeg, MB, Canada)
Heartland Health System (MO)
Highlands Regional Medical Center (FL)
Hoag Memorial Hospital Presbyterian (CA)
Holmes Regional Medical Center (FL)
Holy Spirit Hospital (PA)
Holzer Medical Center (OH)
Hospital for Sick Children (Toronto, ON, Canada)
Hospital Israelita Albert Einstein (Brazil)
Hotel Dieu Hospital (Windsor, ON, Canada)
Huddinge University Hospital (Sweden)
Hurley Medical Center (MI)
Indiana State Board of Health
Indiana University
Instituto Scientifico HS. Raffaele (Italy)
International Health Management Associates, Inc. (IL)
Jersey Shore Medical Center (NJ)
Joel T. Boone Branch Medical Clinic (VA)
John F. Kennedy Medical Center (NJ)
John Randolph Hospital (VA)
Kaiser Permanente (CA)
Kaiser Permanente (MD)
Kantonsspital (AG, Switzerland)
Kenora-Rainy River Regional Laboratory Program (Ontario, Canada)
Kern Medical Center (CA)
King Fahad National Guard Hospital (Saudi Arabia)
King Khalid National Guard Hospital (Saudi Arabia)
Kings County Hospital Center (NY)
Klinični Center (Slovenia)
LabCorp (NC)
Laboratories at Bonfils (CO)
Laboratório Fleury S/C Ltda. (Brazil)
Laboratory Corporation of America (MO)
LAC and USC Healthcare Network (CA)
Lakeland Regional Medical Center (FL)
Lancaster General Hospital (PA)
Langley Air Force Base (VA)
LeBonheur Children's Medical Center (TN)
Lewis-Gale Medical Center (VA)
Libero Instituto Univ. Campus BioMedico (Italy)
Licking Memorial Hospital (OH)
Long Beach Memorial Medical Center (CA)
Louisiana State University Medical Center
Maccabi Medical Care and Health Fund (Israel)
Magee Womens Hospital (PA)
Magnolia Regional Health Center (MS)
Martin Luther King/Drew Medical Center (CA)

Massachusetts General Hospital (Microbiology Laboratory)
Massachusetts General Hospital (Pathology Laboratory)
Mayo Clinic Scottsdale (AZ)
MDS Metro Laboratory Services (Burnaby, BC, Canada)
Medical College of Virginia Hospital
Medicare/Medicaid Certification, State of North Carolina
Memorial Hospital (CO)
Memorial Medical Center (Napoleon Ave., New Orleans, LA)
Memorial Medical Center (N. Jefferson Davis Pkwy., New Orleans, LA)
Memorial Medical Center (IL)
Mercy Health System (PA)
Mercy Hospital (NC)
Mercy Medical Center Des Moines (IA)
Mescalero Indian Hospital (NM)
Methodist Hospitals of Memphis (TN)
Michigan Department of Community Health
Mississippi Baptist Medical Center
Monmouth Medical Center (NJ)
Monte Tabor – Centro Italo - Brazileiro de Promocao (Brazil)
Montreal Children's Hospital (Canada)
Montreal General Hospital (Canada)
Morton Plant Mease Health Care (FL)
Mount Sinai Hospital (NY)
Mount Sinai Medical Center (FL)
MRL Pharmaceutical Services, Inc. (VA)
MRL Reference Laboratory (CA)
National University Hospital (Singapore)
Naval Surface Warfare Center (IN)
New Britain General Hospital (CT)
New England Fertility Institute (CT)
New England Medical Center Hospital (MA)
New York Hospital Medical Center of Queens
New York State Department of Health
NorDx (ME)
North Carolina Laboratory of Public Health
North Mississippi Medical Center
North Shore – Long Island Jewish Health System Laboratories (NY)
Northridge Hospital Medical Center (CA)
Northwestern Memorial Hospital (IL)
Ohio Valley Medical Center (WV)
O.L. Vrouwziekenhuis (Belgium)
Ordre professionnel des technologists médicaux du Québec
Ospedali Riuniti (Italy)
The Ottawa Hospital (Ottawa, ON, Canada)
Our Lady of Lourdes Hospital (NJ)
Our Lady of the Resurrection Medical Center (IL)
Pathology and Cytology Laboratories, Inc. (KY)
Pathology Associates Laboratories (CA)
The Permanente Medical Group (CA)
Pocono Hospital (PA)
Presbyterian Hospital of Dallas (TX)
Prodia Clinical Laboratory (Indonesia)
Providence Health System (OR)
Providence Seattle Medical Center (WA)
Queen Elizabeth Hospital (Prince Edward Island, Canada)
Queensland Health Pathology Services (Australia)
Quest Diagnostics, Incorporated (AZ)
Quest Diagnostics Incorporated (CA)
Quintiles Laboratories, Ltd. (GA)
Reading Hospital and Medical Center (PA)
Regions Hospital
Research Medical Center (MO)
Rex Healthcare (NC)
Rhode Island Department of Health Laboratories
Riyadh Armed Forces Hospital (Saudi Arabia)
Royal Columbian Hospital (New Westminster, BC, Canada)
Saint Mary's Regional Medical Center (NV)
St. Alexius Medical Center (ND)
St. Anthony Hospital (CO)
St. Barnabas Medical Center (NJ)
St. Boniface General Hospital (Winnipeg, Canada)
St. Elizabeth Hospital (NJ)
St-Eustache Hospital (Quebec, Canada)
St. John Hospital and Medical Center (MI)
St. John Regional Hospital (St. John, NB, Canada)
St. Joseph Hospital (NE)
St. Joseph Mercy – Oakland (MI)
St. Joseph's Hospital – Marshfield Clinic (WI)
St. Joseph's Medical Center (CA)
St. Luke's Regional Medical Center (IA)
St. Mark's Hospital (UT)
St. Mary Medical Center (IN)
St. Mary of the Plains Hospital (TX)
St. Mary's Hospital & Medical Center (CO)
St. Paul's Hospital (Vancouver, BC, Montreal)
Ste. Justine Hospital (Montreal, PQ, Canada)
Salina Regional Health Center (KS)
San Francisco General Hospital (CA)
Santa Cabrini Hospital (Montreal, PQ Canada)
Santa Clara Valley Medical Center (CA)
Seoul Nat'l University Hospital (Korea)
Shanghai Center for the Clinical Laboratory (China)
Shands Healthcare (FL)
South Bend Medical Foundation (IN)
Southern California Permanente Medical Group
South Western Area Pathology Service (Australia)
Speciality Laboratories, Inc. (CA)
Stanford Hospital and Clinics (CA)
State of Washington Department of Health
Stormont-Vail Regional Medical Center (KS)
Sun Health-Boswell Hospital (AZ)
Sunrise Hospital and Medical Center (NV)
T.A. Sourasky Medical Center (Israel)
Temple University Hospital (PA)
Tenet Odessa Regional Hospital (TX)
The Toledo Hospital (OH)
Touro Infirmary (LA)
Tri-City Medical Center (CA)

Trident Regional Medical Center (SC)
Tripler Army Medical Center (HI)
Truman Medical Center (MO)
UCSF Medical Center (CA)
UNC Hospitals (NC)
Unilab Clinical Laboratories (CA)
University Hospital (Gent) (Belgium)
University Hospital (TX)
The University Hospitals (OK)
University of Alabama-Birmingham Hospital
University of Alberta Hospitals (Canada)
University of Chicago Hospitals (IL)
University of Florida
University of the Ryukyus (Japan)
University of Texas M.D. Anderson Cancer Center
University of Virginia Medical Center
University of Washington
UPMC Bedford Memorial (PA)
UZ-KUL Medical Center (Belgium)
VA (Dayton) Medical Center (OH)
VA (Denver) Medical Center (CO)
VA (Kansas City) Medical Center (MO)
VA (Martinez) Medical Center (CA)
VA (San Diego) Medical Center (CA)
VA (Tuskegee) Medical Center (AL)
VA Outpatient Clinic (OH)
Vejle Hospital (Denmark)
Virginia Department of Health
Viridae Clinical Sciences, Inc. (Vancouver, BC, Canada)
Washoe Medical Center Laboratory (NV)
Watson Clinic (FL)
Wilford Hall Medical Center (TX)
William Beaumont Hospital (MI)
Williamsburg Community Hospital (VA)
Winn Army Community Hospital (GA)
Wishard Memorial Hospital (IN)
Yonsei University College of Medicine (Korea)
York Hospital (PA)
Zale Lipshy University Hospital (TX)

Contents

Contents (Continued)

Foreword

The primary impetus for this document was the pivotal statement on childhood lead poisoning prevention published by the U.S. Centers for Disease Control and Prevention (CDC) in 1991 which lowered the concentration of lead in blood (BPb) deemed harmful to children from 25 µg/dL to 10 µg/dL (0.48 µmol/L).[1] At this lower BPb level, a widely used biochemical screening test for lead exposure, the erythrocyte protoporphyrin (EP) test, became redundant because EP is insensitive to such low-level lead exposure. The statement recommended that EP screening for lead exposure be discontinued in favor of a direct BPb test. The worldwide impact of the 1991 CDC statement is still being felt as private and public health laboratories continue to respond to the need for inexpensive BPb testing on small capillary blood specimens.

In November 1997, CDC updated its recommendations for screening young children for lead poisoning.[2] The CDC document addresses specific concerns about the extent to which universal screening of all children should be, or can be, implemented. As part of the release of the 1997 document, CDC is providing specific advice and materials to blood lead laboratories (see Appendix C in the CDC document) that complement the guidelines proposed in C40-A. These materials may be downloaded from the CDC's worldwide website at the following URL address:

http://www.cdc.gov/nceh/lead/lead.htm

Because CDC recommendations are often adopted internationally, updated tables from the 1997 document as well as appropriate follow-up actions for confirmed, elevated, blood-lead levels are included here in Appendix C. Information on laboratory accreditation in the U.S. is also provided in Appendix B, along with details of proficiency testing (or external quality assessment) programs for blood lead in the U.S., Canada, and the European Union.

In 1991, established proficiency testing requirements for BPb accuracy were tightened to reflect the improvements in current analytical methodology and the lower concentrations of BPb deemed harmful. Some laboratories using older methods for BPb were unable to maintain proficiency, and were required to improve their analytical method performance. Many were understandably concerned that the analytical technology for making accurate, contamination-free measurements of low levels of lead in capillary blood specimens did not exist. In the years since the 1991 CDC statement was released, it has been shown that current analytical methods can easily measure BPb concentrations below 10 µg/dL with good accuracy and precision. Analytical accuracy continues to improve as evidenced by the performance of participating laboratories in numerous quality assurance and proficiency testing programs. Many laboratories have succeeded in setting up the BPb analysis in-house.

This document addresses many concerns and questions that laboratories have about BPb measurements. Issues of accuracy, interferences, contamination control, and troubleshooting affect all analytical methods. Two analytical methods for blood lead are in routine use at the current time: graphite furnace atomic absorption spectrometry (GFAAS) and anodic stripping voltammetry (ASV). Instrumentation for GFAAS is available from many commercial sources, and a recommended analytical method is described in some detail here. Commercial ASV instrumentation specifically for the BPb analysis is currently available from a single manufacturer. A detailed ASV procedure, which includes use of a proprietary reagent, is provided by the manufacturer. In keeping with NCCLS policy, details of the commercial ASV method are *not* duplicated here; rather, the procedure is summarized, and specific recommendations are given that can help with troubleshooting performance problems. Several other methods are reviewed for their application to BPb measurements, including Delves-cup microsampling flame AAS and inductively coupled-plasma mass spectrometry (ICP-MS). As a relatively high-cost, multielement technique, ICP-MS is unlikely to be used for BPb measurements in most routine situations.

Foreword (Continued)

Analytical chemistry is an evolving science, with new innovative technologies appearing continually. It is quite likely that new, inexpensive, portable technologies for BPb will appear in the future. While it is not possible to address these emerging technologies in the current document, they will no doubt be included in future revisions.

In producing C40-A, the intention of the NCCLS Subcommittee on Lead was to reach a consensus, so an approved guideline can be distributed to laboratories that carry out lead determinations in blood and urine specimens. The subcommittee also intended for the document to be broad in perspective and, thus, an educational resource for laboratorians.

Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to "standard precautions." Standard precautions are new guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80.), [MMWR 1987;36(suppl 2S):2S-18S] and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure, refer to NCCLS document M29—*Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*.

Key Words

Analysis, anodic stripping voltammetry, blood, electrothermal atomic absorption spectrometry, graphite furnace, lead poisoning, quality control, reference materials, urine

Abbreviations

AAS	Atomic absorption spectrometry
APDC	Ammonium pyrrolidine dithiocarbamate
ASV	Anodic stripping voltammetry
BPb	Blood lead
CDC	Centers for Disease Control and Prevention
CRM	Certified reference material
CV	Coefficient of variation (synonymous with RSD)
DL	Detection limit
DMSA	2,3-dimercaptosuccinic acid
EDL	Electrodeless discharge lamp
EDTA	Ethylenediaminetetraacetic acid
EP	Erythrocyte protoporphyrin
ETAAS	Electrothermal atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
FPP	False-positive proportion
GFAAS	Graphite-furnace atomic absorption spectrometry
HCL	Hollow-cathode lamp
ICP-MS	Inductively coupled plasma mass spectrometry

Abbreviations (Continued)

ID	Isotope dilution
IFCC	International Federation of Clinical Chemistry
ISO	International Organization for Standards
IUPAC	International Union of Pure and Applied Chemistry
LC	Liquid chromatography
LDC	Lowest determinable concentration
MIBK	Methylisobutylketone
m_o	Characteristic mass
NHANES	National Health and Nutrition Examination Surveys
NIST	National Institute of Standards and Technology
NRSCL	National Reference System for the Clinical Laboratory
PT	Proficiency test(ing)
OSHA	Occupational Safety and Health Administration
QC	Quality control
RM	Reference material
RSD	Relative standard deviation (synonymous with CV)
SD	Standard deviation
SI	Système International d'Unités
SRM	Standard reference material
STPF	Stabilized temperature platform furnace
TCA	Trichloracetic acid
TIMS	Thermal ionization mass spectrometry
UPb	Urine lead
ZPP	Zinc protoporphyrin

Analytical Procedures for the Determination of Lead in Blood and Urine; Approved Guideline

1 Introduction

The primary aim of this document is to provide clinical laboratories with concise guidelines for measuring lead in blood and urine. It includes specimen collection and measurement by the two principal analytical methods currently in routine use: electrothermal atomic absorption spectrometry (ETAAS), also widely known as graphite furnace atomic absorption spectrometry (GFAAS); and anodic stripping voltammetry (ASV). The document also includes guidelines for quality assurance, quality control, and information on proficiency testing programs and laboratory certification. In developing this guideline the subcommittee recognizes that a single-standard method for GFAAS may not be possible given the complexity of the analytical instrumentation required and the different ways in which manufacturers implement similar features. Furthermore, there is currently only one commercial source of ASV equipment available specifically for BPb measurements. The analyst is free to choose which technique best suits the laboratory's needs, and may modify the recommended procedure to achieve successful analyses. However, whether following the recommended procedure or a modified version, the analyst is responsible for ensuring that the procedure adopted in the laboratory is validated as described under the relevant section.

2 Scope

This document is provided for all laboratories attempting the determination of lead in blood or urine. Laboratories new to the analysis will benefit from the many years of experience accumulated in the laboratories of the committee members, advisors, and observers. A background section on the clinical significance of lead measurements is included to help laboratorians and others understand the context in which these measurements are made. Recommended procedures for collecting blood (both capillary and venous) and urine specimens are given. A detailed analytical procedure is recommended for use with GFAAS equipment. Since an ASV method for BPb is provided by the equipment manufacturer, a detailed procedure is not duplicated here but is summarized. Furthermore, some useful information is included in the ASV section to help users avoid performance problems. Other analytical methods are referenced either within a historical context (e.g., Delves-cup AAS) or with respect to specialized applications (e.g., ICP-MS).

The International Federation of Clinical Chemistry (IFCC) and the International Union of Pure and Applied Chemistry (IUPAC) recommend the exclusive use of the liter as unit of volume when reporting laboratory results using the Système International d'Unités (SI). Many published BPb values are universally reported as mass concentration (unit µg/dL, µg/100 mL) rather than as substance concentration (unit µmol/L). Pediatric urine lead (UPb) measurements are also reported as mass concentration (unit µg/L) and, given the total volume of urine collected, the total lead excreted is calculated and reported (unit µg). In this document, the units for BPb are µg/dL; the units for UPb are µg/L. The IUPAC/IFCC-recommended units of substance (elemental) concentration (µmol/L, µM) are included in parentheses, where appropriate. Conversion factors are given below:

$$\text{blood lead (µg/dL)} \times 0.04826 = \text{blood lead (µmol/L)}$$

$$\text{urine lead (µg/L)} \times 0.004826 = \text{urine lead (µmol/L)}$$

3 Definitions

Please see Section 9 for definitions of **Certified reference material**, **Primary reference material**, **Quality**, **Reference material**, and **Traceability**, which are consistent with those defined in NCCLS document NRSCL8—*Terminology and Definitions for Use in NCCLS Documents.* For complete definitions and detailed source information, please refer to the most current edition of that document.

4 Clinical Significance of Lead Measurements

Lead poisoning is a chronic disease, due to cumulative intake of lead, the course of which may or may not be punctuated by acute symptomatic episodes. If such episodes occur, it is usually during periods of active overexposure. The clinical signs and symptoms of lead poisoning are nonspecific; therefore, a lead measurement, preferably a venous blood lead measurement, is essential for diagnosis. Ancillary tests such as those involving heme precursors (urinary delta-aminolevulinic acid, coproporphyrin, and erythrocyte protoporphyrin) may be helpful in making a diagnosis, but by themselves are inadequate for definitive diagnosis. Clinical symptoms of cumulative lead poisoning generally begin with irritability progressing to loss of appetite, change in personality, increased irritability, and abdominal pain. These manifestations are generally seen starting at blood lead concentrations of approximately 50 µg/dL (2.41 µM). They are, however, easily confused with other diseases that can cause the same symptoms. If the disease is not recognized at this stage, blood lead levels may well increase above 100 µg/dL (4.83 µM), and then the clinical presentation in children is usually with signs of increased intracranial pressure (projectile vomiting, altered state of consciousness, seizures). There is nothing in either the physical or usual clinical or laboratory examinations that would even suggest lead poisoning. In adults, continued exposures at blood lead levels above 50 to 60 µg/dL (2.41 to 2.90 µM) may produce renal failure, decreased cognition, peripheral neuropathy, and gout.

The total body burden of lead may be conveniently divided into four compartments. The residence times of lead in these four compartments are estimated at about: 35 days in blood; 40 days in soft tissues; 3 to 4 years in trabecular bone; and 16 to 20 years in cortical bone. In adults, the disappearance time is largely dependent upon the degree of overall excess exposure. The greater the body lead burden the slower the rate of disappearance from the tissues, including blood.[3,4] Lead in the blood stream reflects the input from three different sources. One component reflects recent assimilation within approximately the past 72 hours; disappearance curves show that there is a second phase lasting for about one to two months, in which the rate of decline is slower; and a third component is contributed by lead entering the bloodstream from bone. These factors should be taken into account when evaluating a series of blood lead measurements. Blood lead measurements, on the other hand, may not be helpful in making a retrospective diagnosis.[5] Injury from lead (for kidneys and CNS) may remain long after blood lead levels have decreased due to distribution and elimination. At present, there is no established way to make a retrospective diagnosis of lead toxicity in a child on the basis of current blood lead alone. Also, acutely high blood lead levels in adults will not produce the expected injury based on relationships known for chronic cumulative exposure.

4.1 Absorption of Lead and Its Internal Distribution Within the Body

Inorganic lead is absorbed by both the respiratory route and alimentary route. Inorganic lead is not absorbed through the skin, although organolead compounds are.[6] Studies in the past have indicated that 40 to 50% of small-particulate lead is absorbed and retained in the lung.[3] With the removal of lead from gasoline during the past decade, respiratory overexposure is much less a problem in the general U.S. population,[7] although industrial exposures to particulate lead can be serious. Adults absorb about 10% of dietary lead. Balance studies in young children show that 40 to 50% of dietary lead is absorbed, and that about one-half the amount absorbed is retained. For a small child an intake in excess of 5 µg per kg body weight per day will result in positive lead balance.[8] Twenty years ago, dietary lead intake often exceeded

this amount but this is no longer the case, as total dietary intake for a two-year-old child has been most recently estimated at 1.9 µg of lead.[7] Lead is distributed throughout the body with the major fraction being absorbed in the bone (95% in the adult and about 70 to 75% in young growing children).[3] The rate of turnover of lead in bone is higher in children than in adults. The two nonosseous organs with the highest lead contents are the liver and the kidney, the organs of excretion of lead. In general, the concentration of lead in other organs is comparable to that found in blood. Approximately 99% of the lead in blood is bound to red blood cells. The remaining 1%, i.e., plasma lead, serves as an intermediate in transporting lead from the erythrocytes to other body compartments.

4.2 Toxic Effects of Exposure to Lead in Children and Adults

Lead affects at least three major organ systems: (1) the central and peripheral nervous systems; (2) the heme biosynthetic pathway; and (3) the renal system. Injury to the renal and cardiovascular systems appear to be closely related. Clinical manifestations differ somewhat between children and adults. In the child, the most serious symptoms are found in the central nervous system as a result of very high exposures. Chelation therapy has reduced the mortality rate and morbidity substantially. Children are much more sensitive than adults to the neurocognitive and behavioral effects of lead, probably primarily for two reasons: (1) children absorb 40 to 50% of dietary lead whereas adults absorb about 10%; and (2) the nervous system develops rapidly in the young child. The blood lead threshold (if there is one) for neurocognitive and behavioral effects is probably lower in children than in adults.[5,9]

In the child, acute renal injury as manifested by the Fanconi syndrome has been associated with blood lead levels of 150 µg/dL (7.24 µM) or higher. The syndrome is reversible.[10,11] Aminoaciduria by itself has been reported at blood lead levels as low as 50 µg/dL (2.41 µM). For some years, evidence has been collected (in Australia only[12]) which indicates that chronic lead poisoning in childhood is a contributor to early adult nephritis. Though three separate studies have been conducted in the U.S., evidence of this has never been identified. The explanation for this difference is not apparent. The Fanconi syndrome is generally not seen in adults; rather the pattern is often one of chronic interstitial nephritis.[13]

In chronic, moderately severe lead poisoning, anemia is commonly found.[14] A decrease in hemoglobin is reported to occur in iron-sufficient children when blood lead concentration exceeds 60 µg/dL (2.90 µM). The comparable value in adults is probably somewhat higher. The anemia is a normocytic, normochromic, well-compensated hemolytic anemia. The degree of hemolysis is insufficient to produce jaundice. Increased erythrocyte destruction is more marked in adults than in children. Increased red cell fragility and decreased osmotic resistance may be observed. Lead's interference in heme biosynthesis is characterized by several unique enzyme blockades causing increased urinary delta-aminolevulinic acid, urinary coproporphyrin, and erythrocyte zinc protoporphyrin. The enzymatic blocks responsible are partial. The basophilic stippling of red cells is due to the presence of mitochondrial fragments, which are not completely destroyed because of the toxic effect of lead on 5-pyrimidine nucleotidase.

One is more likely to encounter peripheral neuropathy clinically in adults. In the distant past, adult encephalopathy has been reported but is rarely seen today. When it occurs in adults, symptoms include dullness, increased irritability, intense headaches, hallucinations, convulsions, and paralysis. The lowest blood lead level at which mean nerve conduction velocities are statistically decreased is about 30 to 40 µg/dL (1.48 to 1.93 µM). Cytologic changes include segmental demyelination and axonal degeneration with concomitant endoneural edema in Schwann cells.[14] These findings are uncommon today but are known to occur if individuals are not adequately protected.

Chronic lead nephropathy is the most important type of injury found in adult populations.[13] Renal dysfunction has been well established in lead workers, many of whom had no history of prior clinical lead poisoning at the time of study. Lead has been clearly demonstrated to produce tubular nephrotoxicity and chronic interstitial nephritis in humans and rodents after chronic exposure.

4.2.1 Reproductive and Developmental Effects

The reproductive toxicity which results from high-dose lead exposure was well known in the last century. In fact the data of the later half of the 1800s led a British royal commission to recommend in 1910 that women henceforth not be employed in the lead trades.[15] This has only changed in the last 30 years, with the return of women to the work force. The obvious effects of lead in the 19th century were stillbirth and spontaneous abortion, which was usually recognized in women with occupational exposure to lead and other clinical manifestations of lead poisoning.[16] In general, spontaneous abortion was an early event. At the present time, we do not know the lowest blood lead at which this may occur, because lead apparently has an effect on the implantation of the fertilized ovum in the uterus. With the advent of human chorionic gonadotropin assays, it is now possible to detect the onset of pregnancy and early fetal loss as early as the first one to two weeks of pregnancy.[10] Sexual dysfunction in the male has not been as closely studied. The studies that have been published, which suggest hypospermia and teratospermia, for example, have been criticized for faulty design. More recently, it has been found in workers employed for more than three years that serum testosterone and free-testosterone index are decreased, at mean blood lead concentrations in excess of 60 µg/dL.[10]

Prospective studies in infants and children, however, have detected some nonfatal effects of moderate increase in lead absorption during pregnancy. A lead-related decrease in the duration of pregnancy, decrease in birth weight, and small-for-gestational-age deliveries have been detected at cord blood lead levels of 15 µg/dL or greater. These findings have not been consistent through all studies. It has been found during the postnatal stage of the prospective studies that the growth rate of infants is slowed. This effect was noted among infants born to women with blood lead concentrations greater than 8 µg/dL during pregnancy. It has also been reported that sustained increases in lead exposure above 20 µg/dL throughout the first 33 months of life are associated with reduced stature.[10] Lead interferes in the formation of active vitamin D, which has an important role in its influence on calcium metabolism. Calcium is under tight homeostatic control in all cells.[10]

Prospective studies on the adverse effects of low-level increase in lead absorption have revealed that there is no association between blood lead concentration at birth and neurobehavioral effects beyond 24 months of age. However, these and other studies suggest that the effects on learning behavior are associated with the degree of lead exposure occurring between 12 and 36 months of age. For example, in the Bellinger study, a significant portion of the variance in cognitive abilities and performance on school test at 10½ years of age is partially predicted by blood lead concentration at 24 months of age.[5,10] The consensus is that lead has an adverse effect on neurodevelopment and cognition. For an increase of 10 µg/dL during the preschool years, an average loss of 2.6 points on the IQ are predicted. While this may seem like a small difference, it is associated with large changes in the percentage of children classified as intellectually gifted or intellectually challenged based on the shift in the IQ distribution.[10] Furthermore, in the few studies that have had the chance to study children with blood leads below 10 µg/dL, some adverse effects on neurodevelopment have been found. Indeed, there may be no blood lead threshold for subtle adverse effects on neurodevelopment. Further detail on this extensively studied topic is beyond the scope of this document but can be found in the literature.[9,10]

4.2.2 Effects of Lead on Blood Pressure and Vitamin D Metabolism

The finding in National Health and Nutrition Examinations Survey II (NHANES II) that lead has a significant effect on both systolic and diastolic pressure has subsequently been confirmed. The overall significance of this in regard to the serious complications of hypertension, such as stroke and coronary heart disease, has not been established. This is a subject under intense investigation at the present time. Current thinking is that alterations by lead in the modulation of the calcium messenger system are what is affecting blood pressure.[13]

It is known that lead interferes with the utilization of iron for the formation of heme. This probably occurs in every cell, although it is best studied in the blood-forming organs. With regard to other organs, it now appears that lead in the kidney interferes with activation of vitamin D 1,2-dihydroxy cholecalciferol, a p450-dependent process.[10]

4.2.3 Mechanisms of Lead Toxicity

We do not yet understand the mechanisms by which lead interferes with calcium functions; however, it is doubtful that lead-calcium interaction is directly responsible for the neurotoxic effects of lead. These changes may be mediated through lead's effects on intracellular calcium homeostasis, or in the brain, for example, by activation of protein kinase C.[17] Lead may interfere with calcium-dependent signal-transduction processes, especially those associated with neurotransmitter function. The latter may be reversible if cellular change has not occurred prior to effective intervention. The effect of lead on blood pressure is more difficult to untangle from lead's well-established effect on the kidney.[13] It does lead to saturnine gout, one of the few characteristics of chronic lead poisoning in the adult. This occurs because of lead's interference in the excretion of uric acid by the kidney. A question here requiring further study is whether significant renal disease can be induced by lead at lower increases in blood lead concentration than those associated with acute symptoms.

4.3 Concentration of Lead in Blood Deemed Safe for Children/Adults

There probably is no such thing as a "safe" blood lead concentration in humans. Indeed, some subtle but statistically significant adverse effects have been found in children on neurodevelopment. Furthermore, the effects of lead on blood pressure can be demonstrated at blood lead levels >10 µg/dL. Rather, 10 µg/dL should be considered the presently "acceptable" or "tolerable" level. It is also an action level according to the statement of the Centers for Disease Control in 1991.[1] Primary prevention should be the goal of all childhood lead screening programs, even though in fact they result at the present largely in secondary prevention. The data from NHANES II and NHANES III[7] give cause for encouragement inasmuch as the average blood lead concentration in the United States has dropped from 15.9 µg/dL in 1978 to 3.6 µg/dL in children in 1991. Thus, the removal of lead in gasoline and the removal of food cans with lead-soldered seams have substantially decreased the overall risk in the United States, leaving old paint as the major cause of lead toxicity in children.

Table 1. Risk Factors Associated with Lead Poisoning

Does your child:

- live in or regularly visit a house built before 1960 with peeling or chipping paint? This could include a day-care center, preschool, or the home of a babysitter or a relative.
- live in or regularly visit a house built before 1960 with ongoing or planned renovation or remodeling?
- have a brother or sister housemate, or playmate being observed or treated for lead poisoning—that is, blood lead level >15 µg/dL (>0.72 µM)?
- live near an active lead smelter, battery recycling plant, or other industry likely to release lead?

The above table and similar tables were proposed in the CDC's 1991 Guidelines for the Prevention of Lead Poisoning.[1] Unfortunately, experience with these questionnaires has been limited and, to a large extent, disappointing. For example, the prevalence of increased lead absorption is perhaps highest in inner-city poor people who rent their homes.[18] Many people do not know when their homes were built, and it is reasonable to expect that the renting population might be unlikely to report reliably on the age of their homes. At the present time, the CDC is reconsidering the approach. It is expected to move to targeted screening. Screening will be targeted to children living in old houses as well as subpopulations of children shown to be at higher than average risk (poor children, children who use folk remedies, and children whose families respond "yes" or "unknown" to a question about the age of their home). Thus, the decision on where to concentrate efforts will be made at the State Health Department level and not through a questionnaire in the field. The 1997 CDC guidelines[2] place more emphasis on diet, particularly in respect to calcium and iron intake, and on better housecleaning techniques. Details on how this will be implemented remain to be worked out.

4.4 Use of Blood Lead Measurements as a Marker of Lead Exposure

Serial venous blood lead measurement is perhaps the best available marker of current and recent lead exposure. It is appropriate for healthcare providers to consult the laboratory in which the measurement is to be made, in order to make certain that the collection and analytic procedures are compatible. Many providers are unaware of the fact that blood specimens may be easily contaminated with environmental lead, unless drawn with the proper needles (stainless steel), syringes (polypropylene), and selected specimen containers. Laboratories will generally provide a guideline to the interpretation of individual blood lead measurements, which are usually modeled after the most recent CDC recommendations. In adults, much higher limits up to 25 or 30 µg/dL (1.21 or 1.45 µM) are allowed in the U.S.A.

Many healthcare providers are unaware of the fact that virtually 99% of the lead in blood is bound to red blood cells and that therefore, whole blood—not serum or plasma—is needed for measurement. Healthcare providers should certainly be made aware of the uncertainty in each measurement. The laboratory should be willing to provide healthcare providers with the results of their performance in blind interlaboratory proficiency programs, as well as the precision and accuracy of measurements made in their own laboratories.

Where sudden changes in blood lead concentration occur, a thorough environmental history will usually reveal the reason. The person might have recently been treated with a chelating agent, in which case the blood lead can drop temporarily and precipitously. There may be recent re-ingestion of lead from an unknown source: Blood lead would rise, but there is always the possibility of contamination of the sample. In any case, unless the clinical history gives a clear indication, the sampling should be repeated. It is important to remember that risk of adverse effects of lead is related to average blood lead concentrations. Concurrent and recent exposures may confound the interpretation. Also, as a rule of thumb, a change in blood lead concentration of 5 µg/dL (0.24 µM) or more should be considered clinically significant, i.e., a real change, whereas smaller changes may not be significant owing in large part to limitations in sampling and analysis.

4.5 Use of Urinary Lead Measurements in the Mobilization Test

In 1963, Emmerson of Brisbane, Australia introduced the calcium disodium EDTA mobilization test as a means of discriminating between those young adults with chronic nephritis with or without a history of lead poisoning during childhood.[18] Those without a history of childhood lead poisoning showed a complete and lower response to this test in 24 hours (< 650 µg/24 h). In those with chronic renal injury apparently due to lead, a four-day collection of urine was necessary, while the peak output often occurred on the second and third day after a single, intravenous infusion of calcium disodium EDTA.[19] Under steady-state conditions, blood lead and $CaNa_2$ EDTA mobilization are well correlated, since both are a measure of available lead in tissues. The test has been used in children to a much lesser extent and

generally without a sound scientific basis. It not recommended in routine care but does remain a potentially useful tool in clinical research.

4.6 *In vivo* X-ray Fluorescence Measurements of Bone Lead

Recently there has been substantial interest in improving the accuracy, precision, and sensitivity of *in vivo* K x-ray fluorescence (K-XRF) for the measurement of lead in bone.[20,21] K-XRF measures immobilized lead in less active bone and reflects cumulative exposure or lifetime pass-through dose. Many of the KXRF details have been worked out. There are, however, few instruments available even on a worldwide basis. It is not anticipated that this will come into routine general clinical use, but it certainly will play an increasingly prominent role in the study of chronic lead intoxication and the build-up of the lead in bone, which constitutes about 95% of the total body lead burden in adults.

4.7 Use of Hair, Sweat, and Other Tissues/Fluids

The use of hair as a sample matrix has been widely investigated. Sample collection is easy and non-invasive, and collected samples are stable indefinitely. However, problems with external contamination, effects of hair treatments, wide interpersonal variation, the absence of a clearly defined normal range, and a lack of correlation with other matrices (e.g., blood) have resulted in widespread rejection of hair as a suitable matrix for lead testing.[22-26] The levels of lead in sweat and nails also do not correlate with blood lead levels and should be considered of no scientific use.[3] Spontaneous urinary lead measurements used to be rather widely used in industry, but generally this is no longer the case inasmuch as precise and generally accurate blood lead measurements are now available. Again, spontaneous urinary lead measurements might be of use in clinical research. Urinary measurements in nontoilet-trained children are extraordinarily difficult to obtain with any consistency, show greater variability, and are very susceptible to contamination. Laboratory tests for basophilic stippling, coproporphyrin, urinary delta-aminolevulinic acid, and x-rays of the long bones are insensitive measures of likely exposures to lead in the U.S. today, though these are abnormal in massive exposures.

4.8 Erythrocyte Protoporphyrin Analysis and Its Use

Erythrocyte protoporphyrin (EP) is the name of the component of red blood cells obtained after treatment (extraction) with ethyl acetate and hydrochloric acid solutions. This form of protoporphyrin is also commonly called "free" erythrocyte protoporphyrin or FEP because the Zn^{2+} ion dissociates from the protoporphyrin IX (PPIX) ligand in acid solution. Following acid extraction, EP is determined using a spectrofluorometer. EP (or FEP) is considered elevated when the concentration is >35 µg/dL, and when the PPIX calibration is standardized using an mmol absorptivity of 241 $L \cdot mmol^{-1} \cdot cm^{-1}$, and >30 µg/dL when using an mmol absorptivity value of 297 $L \cdot mmoL^{-1} \cdot cm^{-1}$. The earlier value of 241 $L \cdot cm^{-1} \cdot mmoL$ was based on the best available information in 1971; however, more accurate and precise procedures indicate that the PPIX absorptivity is about 297 $L \cdot cm^{-1} \cdot mmoL$.[27]

Zinc protoporphyrin (ZPP) represents about 90 to 95% of the total endogenous form of protoporphyrin in human red blood cells. ZPP can be measured directly and simply on a portable hematofluorometer (HF). Because, with the HF, the intrinsic measurement is a ratio of ZPP fluorescence to hemoglobin absorbance, the result is best expressed as µmol ZPP/mol heme. However, most HF instruments in the U.S. normalize this intrinsic measure by multiplying by a single, fixed hemoglobin (or hematocrit) concentration value to make the reporting units comparable, or interpretable, to EP values measured by extraction and expressed in µg/dL whole blood. A detailed discussion of HF calibration is beyond the scope of this document. For more detailed information on HF procedures refer to NCCLS document C42—*Erythrocyte Protoporphyrin Testing*.

The EP test served as the primary screening test for increased lead absorption from 1975 until 1991, when the CDC switched to the blood lead test. With the lowering of the level of concern for blood lead

concentration from 25 to 10 µg/dL, the EP test had lost its utility as a screening test. Although an increase in EP is first detected at a blood lead concentration of about 15 µg/dL in a small portion of the population, it is not until the blood lead reaches 50 to 55 µg/dL[28] that 95% of individuals will have an abnormal response. In addition, blood lead can be related to the adverse neurobehavioral effects of lead; whereas, EP cannot. The EP test has some use as a screening tool for iron deficiency, because an elevated EP indicates impairment in the biosynthetic pathway of heme; though not definitive, however, it may be used in conjunction with red cell indices, serum transferrin or serum iron, and total iron binding capacity. Of these, serum ferritin is considered the most appropriate and sensitive test.

Rises in EP lag behind rises in blood lead by several weeks to two months. The same lag occurs with falling blood lead levels due to decreased exposure or chelation. The reasons for this are the time it takes to accumulate a significant portion of the red-cell population—each of which has accumulated protoporphyrin; and also because of the long red-cell life span. Therefore, EP measurements serve as a short-term index of recent integrated exposure when blood lead levels are >25 µg/dL. In this regard, serial paired blood lead and EP measurements are of considerable value in monitoring the effectiveness of combined medical and environmental management. For more detailed information on the use and interpretation of EP test results, refer to the most current version of NCCLS document C42—*Erythrocyte Protoporphyrin Testing*.

4.9 Reference Intervals for Lead in Blood and Urine

4.9.1 Blood Lead

Studies conducted in the United States indicate that the geometric mean BPb level in the general population has fallen to around 2.3 to 2.8 µg/dL (0.11 to 0.14 µmol/L) and to around 2.7 to 3.6 µg/dL (0.13 to 0.17 µmol/L) in children.[7,29] For public health purposes, BPb concentrations greater than 10 µg/dL (0.48 µmol/L), especially in children, are considered to be lead poisoning. A table of CDC risk classifications for pediatric lead poisoning based upon a confirmed BPb level is reproduced in Appendix C2 (see Section 4.4 on BPb levels for adults).

4.9.2 Urine Lead

There is a paucity of up-to-date published values for normal UPb levels. A 1988 survey of literature data reported a mean value for Pb in urine of 11 µg/L (range 6.3 to 13.0).[30] Normal mean urinary Pb levels from European Community populations have been reported as 14.6 µg/L,[31] and 17 µg/L.[32] In a recent Belgian study, the mean (geometric) normal urinary Pb excretion was reported to be 7.5 µg/g creatinine.[33] Multiplying this by 1.49 g creatinine per day, the mean value for creatinine excretion, yields an excretion rate of 11 µg Pb/day. Reference values for the U.S. population, measured as part of NHANES III (1988 to 1994), indicate that the mean (geometric) UPb concentration is 2.08 µg/L, with the 95th percentile at 6.40 µg/L.[34]

5 Analytical Methods for Lead

5.1 Early Methods for the Determination of Lead

From the early 1940s through to the mid-1960s, the method of choice for blood lead analysis was colorimetry/spectrophotometry using dithizone (diphenylthiocarbazone). This required up to 10 mL of whole blood, complete oxidation using either $HNO_3/HClO_4/H_2SO_4$ or dry ashing, followed by dissolution in acid-citrate, buffering to pH 9.0 in presence of cyanide, and complexation/extraction into a chloroform solution of dithizone.[35-37] Considerable skill was needed to achieve accurate results, not least because the reagent and its lead-complex are photosensitive. Even so, such methods were considered "reliable, sensitive, and free from interference."[38] Automation of the complexation and extraction stages removed some skill requirements and enabled analyses to be done at 10 duplicates per hour.[39,40] The detection limit was 5.4 µg/dL, and the RSD was 8% at 32 µg/dL. Although polarographic methods were available for measuring lead in blood,[41] they were not widely used. The main disadvantages were: large sample volumes, up to 10 mL blood; the necessity for complete oxidation; relatively complex instrumentation; and long analysis times.

5.2 Flame AAS and MIBK Extraction

The low sensitivity of FAAS for lead, approximately 100 µg/dL for 0.0044 A at 217.0 nm, precludes direct analysis of lead in blood. Many workers employed chelation of Pb^{2+}, usually with ammonium pyrrolidine dithiocarbamate (APDC), to eliminate matrix interferences. Berman[42] used trichloracetic acid (TCA) to precipitate proteins from 5-mL volumes of blood and extracted the released Pb^{2+} into methylisobutylketone (MIBK) after buffering the aqueous phase to pH 2.8. An excellent comparison was obtained with analyses by a dithizone method. Hessel[43] also obtained excellent BPb data but with a simpler procedure in which Pb^{2+} was released from 5 mL of blood by hemolysis with Triton X-100® and then extracted directly with APDC/MIBK.

5.3 Delves-Cup FAAS Method

The possibility of measuring lead in microliter volumes of blood/urine appeared in two independent publications in 1968 and 1969: Kahn et al.[44] had developed their Ta-boat technique, and White[45] devised his Pt-wire and Ni absorption tube method. Both techniques involved recording transient absorption signals when dried samples were introduced into an air/C_2H_2 flame. Biological samples produced two signals: one from the radiation/scatter of the combustion products of the sample and the other from lead atoms. These were time-resolved with the Ta-boat technique[44] but not with White's method, which required almost complete prior oxidation of the sample on a spotting tile heated at 450 °C for 20 minutes.

Delves[46] showed that the Ta-boat technique suffered from wide variations in sensitivity, not only between different Ta-boats, but also with different lateral positions within a given boat. These variations did not occur with White's method, since the sample was vaporized into an absorption tube which was effectively a gas cuvette. By combining the better features of both of these techniques, i.e., replacing the Ta-boat with a microcrucible (Ni was used) and vaporizing the sample into a nickel absorption tube, it was possible to measure lead in 10-µL volumes of blood partly oxidized with H_2O_2, in less than five minutes.[46] The method, which had a sensitivity of 100 pg Pb for 0.0044 A at 283.3 nm, an RSD of 4% at the 3-ng level (30 µg/dL), and gave a very good comparison with results of automated colorimetry with dithizone, became known as the Delves-cup method. Between 1971 and 1978 there were some 40 publications describing applications of, or modifications to, the original procedure.[47] The most significant improvements were the use of 217.0 nm rather than 283.3 nm, to minimize molecular absorption interferences, using Al_2O_3 tubes rather than nickel tubes, and integration of absorption signals.[48,49]

It should be noted that a simultaneous continuum background-correction system for AAS was first published by Koirtyohann and Pickett in 1965[50] but was not generally available on commercial instruments sold earlier than about 1972. Consequently, some AAS analytical methods included a procedure for correcting molecular absorption interferences, e.g., repeat analysis at a nearby non-resonance line. The development of simultaneous background-correction systems was an important contribution to analytical accuracy in AAS.

5.4 Anodic Stripping Voltammetry (ASV)

The use of ASV for blood lead analysis was first proposed by Matson et al.[51] in 1971, and was subsequently shown by others to be a valuable micromethod.[52-56] At that time there were two basic procedures for preparation of blood samples for analysis by ASV: acid digestion; and an "exchange" technique in which Pb^{2+} is dissociated from blood proteins using a solution containing $(CH_3COO)_2Ca$, $CrCl_3$ plus Hg^{2+} ions. The main advantage of the exchange technique was speed of analysis, since dissociation was claimed to be complete within three to five minutes, whereas acid digestion of 100 µL blood with $HClO_4$ took about 20 minutes. Both methods of preparation appeared to give reliable data, but the acid digestion method was more precise, i.e., 2.6% at 28 µg/dL[56] compared with 7.8% at 34 µg/100 mL.[55] Several recent studies have compared ASV and GFAAS performance for BPb. They report that the ASV method precision for BPb is poorer than GFAAS, particularly at BPb concentrations below 25 µg/dL.[57,58] Nonetheless, the ASV technique is certainly able to meet current U.S. performance requirements for the BPb analysis.

5.5 Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

Flame AAS methods were not sufficiently sensitive for the direct determination of BPb. When the graphite furnace was introduced in 1970, the greater sensitivity available (about 50 times) finally made it possible to determine Pb in blood with no preconcentration, requiring samples of only µL volumes. The early methods[59-62] showed by 1975 that accurate furnace methods were feasible. The furnace methods of the 1970s were prone to interferences, and deproteinization of the blood or use of matrix-matched blood standards were usually recommended.

The decade of the 1970s was devoted to improving furnace technology. L'vov reminded us in 1970[63] that integrated absorbance signals were preferable to peak absorbance. Lundgren et al.[64] developed the fast heating technique (also called "max power"). Matrix modification was proposed in 1975.[65] By the end of the 1970s, it became apparent that the fast furnace signals were inadequately measured by the slow electronics systems that had been designed for flame AAS, whereas digital electronics were beginning to appear. Autosamplers were developed for commercial furnace AAS at the end of the 1970s. Then, in 1978, L'vov[66] proposed use of a platform on which to deposit the sample, instead of the wall of the graphite tube, to achieve a thermally stable environment during atomization of the analyte. Zeeman background correction, first proposed for use with atomic absorption just before 1970, became commercially available in 1980.

Thus, by the early 1980s, all of the important features of the modern graphite furnace were understood and available for application to analytical problems. These improvements in furnace AAS were consolidated in what has been called the Stabilized Temperature Platform Furnace (STPF).[67,68] Most features of this technology were applied to the determination of BPb by Fernandez and Hilligoss[69] and, following that, by many others.[70,71] The many variations of these methods were summarized and criticized by Subramanian in 1989[72] and by Shuttler and Delves in 1991.[73]

By 1993, Zeeman furnace AAS methods for BPb had evolved that no longer required matrix-matched calibration standards, or deproteinization of the blood samples before injecting them into the furnace.[74] The STPF approach to BPb by GFAAS has now been successfully transferred to a wide range of furnace

equipment including those with continuum and Smith-Hieftje background correction systems, and currently forms the basis of a consensus method for BPb proposed in Section 7.1.

5.6 Mass Spectrometric Methods

5.6.1 Inductively-coupled Plasma Mass Spectrometry (ICP-MS)

It is completely feasible to determine Pb in blood by ICP-MS at well below the 1-µg/dL level usually expected of methods that will meet the modern clinical requirements. ICP-MS is more sensitive than furnace AAS, but it is less tolerant of large matrices. Thus, direct methods by ICP-MS can achieve similar detection limits to furnace AAS, because the increased dilution required is accommodated by the greater sensitivity.

The use of ICP-MS depends upon two characteristics when it is compared with furnace AAS. In certain situations, the greater sensitivity is used when the sample can be freed of some of the matrix. Alternatively, ICP-MS can be used with isotope dilution methods to provide results which, in skillful hands, are judged to be freer of systematic interferences.

There are many papers in the literature that describe Pb determination in blood by ICP-MS,[75-80] though only one claims to do it on large numbers of routine patient samples.[81] Nixon and Moyer[75] used conventional ICP-MS for the simultaneous determination of Pb, As, Cd, and Tl in urine and whole blood using simple dilution of the samples. They use an internal standard to control certain interferences peculiar to ICP-MS. Because their method is a simple dilution for four elements simultaneously, and simple aqueous standards are used, they report that it is twice as fast as furnace AAS.

The source of Pb in poisoning cases was determined by Delves and Campbell[76,77] using isotope ratios found by ICP-MS. Pb from different geological sources displays a characteristic ratio of the several isotopes depending on the parent atoms from which it decayed. Thus the isotope ratio of Pb in a poisoned child could be matched to a particular paint in the child's environment. They showed that the Pb isotope ratio in groups of people in a particular locality reflected the ratio of the isotopes in the drinking water of that locality. ICP-MS was used as a detector for liquid chromatography (LC) by Gercken and Barnes[78] in studies of the biological binding sites for Pb. They also studied Pb speciation in biological materials using LC and ICP-MS.

There are numerous other references in the literature cited, but the material presented here provides an insight into the several ways that ICP-MS is used for the determination of Pb in various biological materials. The opportunity for the future is very great.

5.6.2 Thermal Ionization Mass Spectrometry (TIMS)

TIMS is an analytical technique in which a highly purified, isotopic solid material, lead in this case, is thermally ionized in a solid-source mass spectrometer. The Pb^+ ions are accelerated into a mass analyzer and thence to a detector. The mass analyzer separates the ions on the basis of their mass-to-charge ratio. Traditionally, the mass analyzers for TIMS have been magnetic sectors; however, thermal ionization quadrupole instruments have also been developed.

TIMS has been used and refined for lead analyses over many years and is the technique of choice for geologists who wish to determine the absolute age of rocks using the U/Pb clock. Among the advantages of TIMS are high sensitivity and high precision; however, the quantitation of lead and other element concentrations can only be done using isotope dilution analysis (ID). This is because the ionization efficiency of lead during the heating process is a complex function of many variables such as temperature, time, and the chemical form of the sample; and these conditions are not easily reproduced. Typically, ID-

TIMS can achieve levels of precision on the order of 0.05%, while for ID-ICP-MS it is around an order of magnitude worse. This is due to the nonspecificity of the ionization process in the plasma.

5.6.3 Isotope Dilution Mass Spectrometry (ID-MS)

When the considerable sensitivity of the mass spectrometer for individual isotopes is coupled with the isotope dilution technique, the resulting method, ID-MS, is considered a fundamental reference method. ID-MS may be used with both ICP-MS instrumentation or with TIMS instrumentation. ID-TIMS is more precise and generally the more sensitive of the two, but is also more expensive and complex.

An example of the use of ICP-MS with isotope dilution for accurate determinations of Pb in blood is provided by Paschal et al.[79] In isotope dilution analysis, a precisely known amount of a rare isotope of the element is spiked in the sample. This is usually done prior to any handling of the sample, and the spike is treated somewhat as if it were an internal standard. In the Paschal et al.[79] method, the blood aliquots were digested in HNO_3 in a microwave oven.

Advantage has been taken of the increased sensitivity of ICP-MS for these biological matrices by other workers. Bowins and McNutt[80] used isotope dilution ICP-MS to measure Pb in human plasma, where the levels are very low. To handle the problem of a difficult matrix, they introduced the sample in a graphite furnace and separated the vaporization of the analyte from that of the bulk of the matrix. They report a detection limit of about 3 pg/L. They found the Pb content of a composite normal human plasma sample to be 1.3 µg/L.

ID-TIMS has been one of the primary technologies responsible for the improved accuracy of inorganic analytes in clinical chemistry standards[82-84] and has substantially aided the standardization and accuracy concerns of the National Reference System for the Clinical Laboratory.[85] It has come to be accepted as one of the few definitive methods capable of delivering proven and demonstrated accuracy during the certification process of a primary reference material.[85-87] ID-MS is a relatively complex method that is both accurate and robust, because the chemical manipulations can all be done on a mass basis, while the mass spectrometer measurements are isotope ratios rather than absolute isotope intensities. This latter property of ID-TIMS virtually eliminates systematic biases such as incomplete recoveries during sample digestion and separation.

Lead is one of the elements of the periodic table that can show considerable variation in the isotopic abundance of its four isotopes. ID-MS thus requires two separate analyses to characterize a sample fully. First, an aliquot of the specimen is measured for its isotopic composition. A second aliquot is then spiked with an enriched isotope of lead, generally ^{206}Pb, and the appropriate isotopic ratio is measured. Although the isotopic measurements from TIMS always show a bias due to isotopic fractionation during the ionization process, the fractionation arises due to kinetic effects and can be controlled and corrected.

Researchers, such as C. Patterson,[88] have repeatedly shown that they can quantitatively determine picogram amounts of lead because of the high sensitivity of TIMS. Source apportionment studies of lead poisoning using lead isotopic ratios benefit greatly from the high precision of the TIMS measurements.[89] However, the cost of the solid-source mass spectrometers and the specialized clean labs necessary for the chemical separation of lead, together with the relatively low sample throughput, mean that TIMS will remain a research tool for BPb measurements.

5.7 Future Approaches to Blood Lead Measurements

Several technological advancements in blood lead determination have occurred in recent years. These advancements are very diverse and include updated and enhanced older technologies as well as new analytical technologies. For example, several electrochemical methods for blood lead have been developed that use analog and digital electronics coupled with screen-printed microelectrodes to perform

anodic stripping voltammetry (ASV) and/or potentiometric stripping analysis (PSA).[90-93] These methods are currently being implemented in commercially marketed hand-held analytical devices for measuring lead and other trace metals in environmental samples. Methods have also been described for blood lead that are based on atomic absorption spectrometry but which utilize a tungsten filament atomizer.[94] Such an approach would make a portable instrument dedicated to the blood lead analyses quite feasible. In another portable spectroscopic approach, a microwave-coupled plasma, with direct sample addition to a filament, was used in atomic emission mode for the determination of lead in blood.[95,96] Yet another approach has been the novel use of polymeric materials, coupled to a sensor, that "stretch or shrink" upon addition of lead.

The development of these novel technologies for the measurement of lead in blood has been strongly supported and encouraged by the CDC. The aim of the CDC program is the development of new novel ideas for blood lead measurement that could lead to low-cost dedicated instruments that are rugged, portable, easy-to-operate, and allow for immediate testing results (i.e., results that are available within a few minutes). Such instruments would be expected to measure <10 µg/dL and, at a lower cost per test than current lab-based methods. It is expected that these new technologies will permit screening for blood lead in locations such as health fairs, lead clinics, in-the-field surveys, etc. Such devices may also permit immediate retesting of children who are determined to have elevated blood lead results. Another significant application of these new portable technologies is in countries which either cannot afford laboratory-based methods, or which have no infrastructure for rapid transport of blood samples to a centralized laboratory facility.

6 Procedure for Collecting Blood and Urine for Lead Determination (see Section 10.3)

For procedures to evaluate the potential for lead contamination in collection materials and supplies, refer to Appendix A.

6.1 Procedure for Collecting Capillary Blood Specimens

The following procedure is specific for the measurement of lead in capillary blood collected for childhood screening purposes. Refer to the most current editions of NCCLS documents C38—*Control of Preanalytical Variation in Trace Element Determinations;* and H4—*Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture,* for measuring other analytes.

6.1.1 Background

The high potential for lead contamination of capillary blood samples during collection has been known for some time.[97-99] Some workers have suggested using special steps to minimize Pb contamination errors during sample collection. These include thoroughly scrubbing (with soap and then alcohol) of the hand and finger to be punctured[100,101]; using dilute (0.3 M) nitric acid[62,102]; or using a barrier spray, e.g., silicone, to reduce errors due to lead contamination.[99,103]

Several recent studies[104-106] have examined the performance of capillary BPb screening by comparing results with venous BPb levels obtained simultaneously. These studies have also evaluated the effectiveness of a silicone barrier spray in reducing contamination errors, and each has found that silicone offers no advantage in reducing contamination. Thus, the only value in using a silicone barrier spray is that it promotes blood beading, which facilitates blood transfer into a glass capillary tube. However, with the availability of plastic microcollection devices, this advantage is no longer important. These studies also conclude that, with careful attention to cleaning the child's hands, and the use of lead-free materials, very reliable screening results are possible. For example, Rainey et al.[107] estimated a false-positive proportion (FPP), defined as the number of false-positive errors divided by the number of children

screened, of 7.6% at 15 µg/dL; Schlenker et al.[106] report a FPP of 1% to 5% at 20 µg/dL; and Parsons et al.[104] report a FPP of 4% at 15 µg/dL, and only a single false-negative result in a study of some 500 cases. In the latter study, more than half of the false-positive BPb errors were attributed to a single collector, who did not wash the child's hand before puncturing the finger.[104] ***Clearly, thorough hand washing is a critical component of a good BPb screening technique.***

Several types of plastic microcollection containers (typical fill volumes 100 µL – 1 mL) are available for collecting and transporting capillary blood. These plastic microcontainers are safer than glass microhematocrit tubes, which can snap when removing the blood specimen, causing fingerstick injuries to laboratory staff. While whole capillary blood is acceptable for screening purposes, venous blood is the only reliable and acceptable specimen for the confirmation, diagnosis, or medical management of lead poisoning.

The use of dried blood spots on filter paper for blood lead measurements (FP/Pb) was first proposed as a screening test almost 30 years ago.[108] Several investigations have reported on the development of FP/Pb measurement technology.[109-121] Recent investigations have reported the successful use of FP/Pb methods for the screening and identification of patients with blood lead levels above 10 µg/dL.[122-129]

6.1.2 Procedure

The following recommended procedure for collecting pediatric capillary blood specimens for lead testing is based upon a consensus of experience from several U.S. State Health Departments.[130-134] It has been validated in several studies[104-106] and is also recommended by the U.S. Centers for Disease Control and Prevention.[1, 2]

Ensure that collection tubes, lancets, and needles have been checked for lead contamination as described in Appendix A. The materials needed to carry out this procedure include:

- Soap.
- Alcohol swabs. If a surgical or other disinfectant soap is used, alcohol swabs can be eliminated.
- Sterile cotton balls or gauze pads.
- Latex or vinyl disposable examination gloves (powder-free preferred).
- Lancets. The type of lancet used is largely a matter of personal preference, so long as sterility is guaranteed.
- Capillary microcollection containers. The laboratory should be consulted to ensure that an appropriate-size tube is used, and that the anticoagulant, either EDTA or heparin, is compatible with the laboratory's analytical methodology. **NOTE**: EDTA is normally preferred.
- Adhesive bandages.
- Disposable bags suitable for medical waste and containers for sharps. Bags and containers for medical waste and sharps, respectively, should be clearly identified as such.
- Storage or mailing containers, if needed. If specimens require shipment, follow appropriate regulations for shipping clinical specimens (e.g., U.S. Postal Service, Dept. of Transportation).
- Laboratory coat and protective glasses.

Materials used in the collection procedure that could contaminate the specimen (for example, blood containers and alcohol swabs) must be free from significant lead contamination. (See Appendix A for guidance on contamination testing and Section A1.9 for interpretation of results and definition for "significant lead contamination.") ***Before selecting equipment for use in blood collection, consult with the laboratory about its requirements***. In many cases, the laboratory will recommend or supply suitable collection equipment, and should assure that they are free of significant lead contamination. Some manufacturers supply collection materials that are pretested for lead content.

6.1.2.1 Preparing for Blood Collection

All personnel who collect specimens should be well trained in and thoroughly familiar with the collection procedure. The skill of the collector will greatly influence the specimen quality. All equipment should be within easy reach. The environment should be clean, secure, and as nonthreatening to the child as possible. Any necessary consent should be obtained before specimen collection begins, and the procedure should be explained to the child and the parent or guardian. Used materials should be discarded into appropriate waste containers, suitable for medical waste, immediately following use.

Every effort should be made to collect a venous specimen for diagnostic/confirmatory purposes. If all efforts fail, the laboratory may accept a second capillary blood specimen, if it can confirm that the specimen was collected using meticulous technique to prevent contamination. However, elevated BPb on capillary specimens must be considered presumptive and must be confirmed with venous blood. *Under no circumstances should chelation therapy be based on a capillary blood lead result.*

6.1.2.2 Preparing the Finger for Puncture

NOTE: Puncturing the fingers of infants less than one year of age is not recommended. Puncturing of the heel or toe may be more suitable for these children. (Refer to the most current edition of NCCLS document H4—*Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture*).

Collection personnel should wear examination gloves whenever the potential for contact with blood exists. If the gloves are coated with powder, they should be rinsed with water before handling blood collection materials or touching the child's hand.

The child's hands should be thoroughly washed with soap and then dried with a clean, low-lint towel. Plain, unprinted, nonrecycled towels have been found to be suitable.[131] If desired, a brush can be used for cleaning the finger; brushing during washing can increase blood circulation in the finger.[133] Once washed, the finger must not be allowed to come into contact with any surface, (e.g., door, chair, counter-top, or even the child's other fingers), except the gloved hand of the person performing the procedure.

The finger to be punctured (often the middle finger) must be free of any visible infection or wound; it should be massaged to increase circulation before being punctured with the lancet. This can be accomplished during or after washing.[130,133]

6.1.2.3 Procedure for Preparing the Child's Finger

(1) Select examination gloves. If necessary, rinse them to remove powder.

(2) Wash the child's hands thoroughly with soap and water, and then dry them with an appropriate towel.

(3) Grasp the finger that has been selected for puncture between your thumb and index finger, with the palm of the child's hand facing up.

(4) If not done during washing (see preceding notes), gently massage the fleshy portion of the finger.

(5) Clean the ball or pad of the finger to be punctured with the alcohol swab. Dry the fingertip using the sterile gauze or cotton ball.

6.1.2.4 Puncturing the Finger

After the finger is ready, the puncture and subsequent steps of forming a drop of blood and filling the collection container should be performed quickly and efficiently, since any delay can make collection more difficult. (For example, the blood may clot or the child may resist.) Several types of lancets are suitable for puncturing children's fingers. They range from small, manual lancet blades and spring-loaded assemblies to disposable, self-contained units. The latter are particularly attractive, since the blade is automatically retracted into the holder after use, thus reducing the risk of self-injury. Many devices are available with a selection of puncture depths suitable for very small children or adults. Regardless of the lancet used, the puncture should be made swiftly and cleanly and should be deep enough to allow adequate flow.

The site of the puncture should be slightly lateral to the ball of the finger. This region is generally less calloused, which makes puncturing easier and possibly less painful.[133] The first drop of blood contains tissue fluids that will produce inaccurate results; it should be removed with a sterile gauze or cotton ball.[130,133]

Blood that runs down the finger or around the fingernail is no longer suitable. Blood flows better if the punctured finger is kept lower than the heart. Inadequate blood flow can be improved by gently massaging the proximal portion of the finger in a distal direction, then pressing firmly at the distal joint of the punctured finger (restricting blood flow out of the fingertip) and *gently* squeezing the sides of the fingertip. Avoid excessive squeezing or "milking," which will cause tissue fluid to be expressed, compromising specimen integrity.[132,133]

6.1.2.5 Procedure for Puncturing the Finger and Forming Drops of Blood

(1) Grasp the finger and quickly puncture it with a sterile lancet in a position slightly lateral to the center of the fingertip.

(2) Wipe off the first droplet of blood with the sterile gauze or cotton ball.

(3) If blood flow is inadequate, gently massage the proximal portion of the finger and then press firmly on the distal joint of the finger.

(4) A well-beaded drop of blood should form at the puncture site.

(5) Do not let the blood run down the finger or onto the fingernail.

6.1.3 Filling the Collection Container

The proper procedure for filling and capping collection containers is somewhat specific to the container used. As a general rule, contact between the skin and the container should be avoided. To prevent specimen clotting, blood must be thoroughly mixed with the anticoagulant after filling the container. Depending on the container and anticoagulant used, the agitation needed can range from gentle rocking to vigorous shaking. Some procedures call for the collection container to be rotated during filling so that anticoagulant will be distributed quickly through the sample.[134] For collectors already familiar with fingerstick blood collection for other purposes, e.g., hematocrit, CBC, etc., there may be a tendency not to

agitate the blood sample too vigorously lest the blood cells rupture. This is not an issue for BPb, since the prevention of clotting prior to analysis is more important than preserving erythrocyte integrity.

To facilitate blood flow, many procedures call for the collection container to be held nearly horizontal, with a slight downward angle. Blood flow into the container should be uninterrupted to avoid air bubbles in the specimen. Microcontainers come with appropriate caps, and these should be applied immediately following collection. Consulting with the laboratory and knowing the manufacturer's recommendations are important to ensure specimen integrity and suitability for analysis.

6.1.3.1 Procedure for Filling the Collection Container

(1) Continuing to grasp the finger, touch the tip of the collection container to the beaded drop of blood.

(2) Draw the blood into the container, maintaining continuous flow of blood.

(3) When full, cap or seal the container as appropriate.

(4) ***Agitate the specimen to mix the anticoagulant thoroughly with the blood***.

(5) Check that the container is properly labeled, and place it in an appropriate storage area.

(6) Stop the bleeding and cover the finger with an adhesive bandage. Bleeding should stop very quickly. If bleeding is slow to stop, apply pressure to the puncture site with sterile gauze or a cotton ball. If bleeding continues after three to five minutes of applying pressure, consult a physician.

6.2 Procedure for Collecting Venous Blood Specimens

Refer to the most current edition of NCCLS document H3—*Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture* for a more detailed procedure.

6.2.1 Required Supplies

The materials needed to carry out this procedure include:

- prescreened EDTA blood-collection tubes (0.5 mL or greater);
- 20- to 23-gauge multisample needles or butterfly needles;
- needle/tube holders if required;
- gauze sponges;
- alcohol wipes (70% isopropanol);
- bandages;
- marker pen;
- rack or box to support tubes;
- optional: “cool packs” or wet ice to maintain cool temperatures in situations where specimens are to be shipped to or from hot climates;

- disposable paper towels or plastic-backed “diaper” counter liners;
- biohazard container for disposal of contaminated supplies after use;
- tourniquets;
- powder-free latex or vinyl disposable gloves; and
- laboratory coat and protective glasses.

6.2.2 Preparation

Assemble all required blood collection and approved needle-disposal materials in advance (see Section 6.2.1, Required Supplies). Wear disposable latex or vinyl gloves, laboratory coat, and eye protection.

6.2.3 Blood Collection

Draw the blood from an antecubital vein or from some other convenient vein. Apply the tourniquet to locate the antecubital vein, then release it while the venipuncture site is being cleaned. If the subject's arm is extremely dirty, wash the antecubital area thoroughly with soap and water and dry it with a disposable towel. Then, swab the venipuncture site with alcohol and allow it to dry. Reapply the tourniquet, reconfirm the vein location, and perform the venipuncture with the bevel of the needle facing upward. (Care should be taken to avoid protracted probing for vein location.) Collect blood using either a regular multisample needle or a “butterfly” needle in combination with a 0.5-mL or greater EDTA specimen collection tube, filling the tube as completely as possible. (**NOTE**: Some specimen tubes may be the same physical size with varying nominal volumes.) Release the tourniquet before the needle is removed from the vein. Prolonged application of the tourniquet should be avoided to minimize hemoconcentration. Invert the tube *gently* about ten times to allow thorough mixing of blood with the anticoagulant.

Use a dry gauze pad to apply pressure when removing the needle, since a wet pad could result in drawing fluid into the collection tube. After removing the needle, have the subject apply pressure firmly over the puncture site. If the subject is a child not old enough to perform this task, maintain pressure on the site. Reexamine the puncture site to verify that any residual bleeding has ceased after five minutes, then apply a bandage as a precaution. Label the tube with the patient's name or other identifying parameter. Dispose of all needles and contaminated wastes properly in the biosafety container.

6.2.4 Safety Note

CDC and NCCLS recommend as good laboratory practice that all blood specimens, used needles, etc., should be treated as though they were infectious for HIV and hepatitis B virus. All used needles should be placed in puncture-resistant containers; then, along with used gauze and other blood-contaminated items, they should be autoclaved prior to disposal. Use of disposable gloves when collecting and processing blood is also required. (See “CDC recommendations for preventing transmission of infection with human HTLV III/LAV in the workplace.” MMWR 1985;34:682-685, and the most current edition of NCCLS document H4—*Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture*).

6.2.5 Transportation of Specimen (see Sections 6.1.1,10.3.3 to 10.3.5)

If the collection site is in the field, or some distance from the testing laboratory, store the specimens securely in an upright position in a box or a rack. When collection is performed in the field, place the box or rack in an insulated cooler or polystyrene shipper, with either “cool-packs” or wet ice in a transparent

plastic storage bag sealed along the edges of the opening, to maintain the specimens at a cool, but not freezing, temperature during storage. Blood lead specimens are very stable for long periods of time if stored at 2 to 8 °C.[135]

Include all test requisition information such as subject age, sex, address, etc. (see Appendix C1) as required by the analyzing laboratory, and ensure that specimen tube identification matches the accompanying documentation. ***Do not*** open specimen tubes prior to shipment. If additional analyses other than lead (such as erythrocyte protoporphyrin, CBC, or hematocrit) are also planned for the EDTA-whole blood specimen, it is imperative that the blood lead assay be performed first, followed by other tests, to prevent contamination of the specimen. As an alternative, once specimens are received at the laboratory, they may be subdivided in a very clean area, such as under a laminar-flow hood or a biological safety cabinet, into prescreened high-density polypropylene cryovials, and the some of the vials may then be frozen for later analysis. (Lead and EP [by extraction] may be performed on frozen whole blood.)

Specimens may be shipped to the analyzing laboratory for blood lead measurement by placing them in an approved container that will prevent breakage during transit. Include all test requisition information, and check that specimen identification matches the accompanying documentation.

Most laboratories accept specimens shipped at ambient temperature, and most blood lead proficiency testing programs routinely ship their specimens at ambient temperature, too. However, it should be noted that blood specimens shipped during periods of hot weather, particularly in warmer climates, might be adversely affected by prolonged exposure to elevated temperatures. In such situations, it would be prudent to place specimens boxes in a polystyrene-insulated cooler with "cool-packs" to maintain a cool, but not freezing, temperature during shipment.

6.3 Procedure for Collecting Urine Specimens

The UPb test is used by some clinicians to monitor lead body burden following therapeutic mobilization tests, such as $CaNa_2$ EDTA,[1] or for evaluating occupational exposure to organolead compounds such as tetraethyllead (TEL).[102] Urine is considered an acceptable specimen for (a) establishing Pb excretion rates; (b) screening adult workers exposed to lead; and (c) for mobilization tests. However, it is essential that meticulous attention be paid during the collection of urine specimens to avoid contamination errors.

6.3.1 24-Hour Collection

If definitive data on urinary excretion of lead is required, a 24-hour urine collection yields the preferred specimen. Urine collection should be arranged away from the suspected exposure site. If workplace exposure is suspected, the collection should be taken during off hours, preferably on the weekend, away from the work environment (to avoid contamination from work clothing or atmosphere). Refer to the most current edition of NCCLS document GP16—*Routine Urinalysis and Collection, Transportation, and Preservation of Urine Specimens* for more detailed directions on collecting urine specimens from adults and children. The procedure is summarized below:

(1) Provide the subject with a 1- to 2-L, lead-free,[a] wide-mouthed voiding container for direct urine collection. Metal or porcelain collection containers must be avoided. ***Do not*** use metal urinals or pans.

[a] Lead-free is interpreted as either (a) acid-leached/washed, or (b) lot-tested to ensure that the containers are free from significant lead contamination. (See Appendix A for guidance on contamination testing and Section A1.9 for interpretation of results and definition for "significant lead contamination.")

(2) Measure the volume of the well-mixed 24-hour specimen in a lead-free, 2-L measuring cylinder.

(3) Transfer a 50- to 100-mL urine aliquot from step (2) into a lead-free, plastic, screw-capped bottle. ***Do not*** use the plastic jars with metal lids typically found in hospital settings or urinalysis laboratories, unless they have been specifically tested for Pb and found to be lead-free.

(4) ***Do not*** add any preservatives to urine specimens, such as HCl or HNO_3, because there is increased potential for contamination, and acidification is unnecessary for UPb specimens.

NOTE: A quantitative 24-hour collection is preferred for clinical research purposes.

6.3.2 Random Urine

While a 24-hour urine collection is preferable to a random spot urine, the latter is sometimes used for screening (arsenic, mercury, or lead) in cases of occupational exposure. The simultaneous measurement of urinary creatinine, reporting results as µg Pb/g creatinine may assist with the validity of the result interpretation.[136] For example, [(µg/L lead x 100) / (mg/dL creatinine)] = µg lead/g creatinine.

Urine can be collected in a random manner by using a suitable plastic container with a plastic lid. Typically, 130-mL (4.5-oz) sterile containers are available in a physician's office or in a hospital setting. Before these containers are used for collection, their suitability for use in lead determinations should be validated.

The entire urine spot collection can be delivered to the laboratory or a small portion (5 mL) can be poured off into a plastic screw-cap vial and sent to the laboratory. Again, the sample need not be acidified.

6.3.3 Eight-hour Timed Collection (Calcium Disodium EDTA Lead Mobilization Test)

The eight-hour timed collection for the $CaNa_2EDTA$ provocation or mobilization test is designed to provoke a brisk lead diuresis in which the total amount of lead excreted over an eight-hour period is measured. This is normally carried out on an out-patient basis.

All materials used to collect urine and transport the specimen to the laboratory must be checked and certified as lead-free. Therefore, it is the responsibility of the laboratory performing the analysis to provide the appropriate supplies and ensure that they are lead-free. The supplies can include a 1-L, acid-washed, plastic urine collection container and a 5- to 10-mL plastic tube or other device for transferring the urine sample aliquot and shipping it to the analyzing laboratory.

The procedure for collecting, transporting, and storing an eight-hour timed urine specimen is summarized below.

(1) Have the patient excrete urine into a plastic, acid-washed container over an eight-hour period. Record the total volume (mL) of urine collected along with the dose of $CaNa_2EDTA$ (mg).

(2) Immediately after urine collection, mix well, and transfer a 5- to 10-mL aliquot into a plastic, leak-proof tube for transport to the analyzing laboratory. This may be easily accomplished using a purpose-designed plastic device for transporting samples for urinalysis.

(3) ***Do not*** add any preservatives to urine specimens, such as HCl or HNO_3, because there is increased potential for contamination, and acidification is unnecessary for UPb specimens.

(4) Urine samples may be transported to the laboratory at ambient temperature using approved shipping containers for etiologic agents.

(5) Upon receipt, refrigerate urine samples at 4 °C or frozen until the analysis can proceed.

NOTE: Recently the FDA has approved the use of meso-2,3-dimercaptosuccinic acid (DMSA) or "Succimer" for the treatment of lead poisoning in children with BPb levels greater than 45 µg/dL. In most cases, UPb is not monitored during or after treatment with DMSA.

7 Procedure for Pb in Blood and Urine by Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

7.1 Principle of Measurement and Method Summary

Lead is determined in blood and urine by graphite furnace atomic absorption spectrometry (GFAAS), which is also known as electrothermal atomic absorption spectrometry (ETAAS). Measurement is based on the amount of light absorbed at 283.3 nm from either a hollow-cathode lamp (HCL) or an electrodeless discharge lamp (EDL) source by ground state atoms of lead in the gas phase. Blood samples, blood-based quality control materials, and aqueous Pb standards are diluted 1 + 9 with a solution containing ammonium dihydrogen phosphate modifier, nitric acid, and Triton X-100®. The lead content is determined using a graphite furnace atomic absorption spectrometer equipped with a Zeeman, Continuum, or Smith-Hieftje background correction system, and operated under stabilized temperature platform furnace (STPF) conditions.

The method recommended here is based upon that described most recently by Parsons and Slavin[74] for use with a transversely heated furnace system and a longitudinal Zeeman-effect background correction system. It calls for rapid-heating platform atomization with integrated absorbance measurements, and the use of phosphate modifier with Triton X-100®. The method draws heavily upon the work of others including Pruszkowska, Carnrick, and Slavin,[137] and Miller, Paschal, Gunter, et al.[71] The method has been successfully transferred to a simpler furnace arrangement that utilizes continuum background correction,[138] and to furnace AAS equipment from other manufacturers utilizing Zeeman, Continuum, and Smith-Hieftje background correction systems.[139,140]

There is a limited amount of information available on analyzing urine specimens for lead.[141-144] Requests for UPb analysis are now so infrequent that most laboratories have limited experience. The situation for the analytical laboratory is complicated by the lack of certified urine-based reference materials with lead concentrations that are relevant to clinical experience. Few commercial control materials exist, and the target values have large uncertainties.[145] The STPF-based procedure suggested below is similar to the approach for BPb, and provides for either aqueous or matrix-matched standards for calibration. It has been validated in interlaboratory studies but only for the transversely heated furnace system equipped with longitudinal background correction.[145]

Lead contamination must be minimized throughout all analytical procedures, but especially during the specimen collection. All materials used for collecting and processing clinical specimens should be screened for possible lead contamination. All work should be performed under clean conditions and analytical samples protected from dust.

7.2 Instrumentation Requirements

7.2.1 Instrumental Requirements

The following points should be considered:

- Atomic absorption spectrophotometer equipped with electrothermal atomization, background correction system, autosampler, and controlled fast heating during atomization.

- High-purity pyrolytically coated graphite tubes with solid pyrolytic graphite L'vov platforms.

- Argon gas tank (zero grade - 99.996% purity), equipped with an approved gas regulator.

- General Ranges for Instrument Settings:

  Analytical wavelength: 283.3 nm.
  Peak measurement: integrated absorbance.
  Integration time: 2 to 5 seconds.
  Band pass: 0.5 to 1.0 nm (low).
  Calibration: Linear (zero intercept).

7.2.2 Optimizing the Furnace Program Parameters

It is not possible to give a fixed set of furnace program parameters for BPb that can be used with all equipment from any instrument manufacturer. Differences in furnace design and the various ways in which manufacturers implement important features mean that some degree of optimization will be necessary before proceeding with the analysis. The major AAS instrument companies may provide recommended furnace parameters for the determination of lead in blood, but the analyst may be required to confirm/validate these parameters in order to optimize the analysis. Before tackling any optimization, the analyst should consult the manufacturer's technical product staff to determine whether these parameters have been optimized by others.

In the event that there is no previous experience to draw on for a particular furnace make or model, the analyst should conduct the following optimization studies.

7.2.2.1 Deposition on the Platform

Previous experience with a wide range of furnace instrumentation using 12-µL injections of 1 + 9 diluted blood on the platform has been quite successful.[74] The amount of blood matrix deposited is small enough to be removed during the run cycle without experiencing carbonaceous build-up. If carbonaceous build-up is observed after several firings, it may be necessary to add an oxygen ashing step to the furnace program. The use of an oxygen ashing step under these circumstances has been described for the BPb determination using GFAAS.[70] Care should be taken when using oxygen in an ashing step to avoid high temperatures which will destroy the graphite tube. It is also recommended that the tube be purged with argon at 900 °C before atomization in order to desorb oxygen.

It is critical to ensure that the sample is quantitatively deposited on the L'vov platform. Most often this is ignored. The injection tip should not touch the furnace wall or platform directly. Adding a few milliliters of ethanol or isopropyl alcohol to the autosampler wash solution to rinse the tip after sample deposition can prevent a build-up of organic debris on the injector tip and reduce bad injections due to sample "hang-up." The addition of a little Triton-X 100® (0.005%) to the wash solution also helps to reduce build-up.

7.2.2.2 Drying

The sample must be dried carefully and completely, without boiling or sputtering. Watching the sample (both aqueous standard and blood) dry is considered mandatory. The use of a small mirror (e.g., dental mirror) is useful for this purpose. If the furnace has a "hot-inject" feature, i.e., a preheated platform, this can be used to facilitate sample drying and reduce the hold times in this stage of the program. It is difficult to define a protocol for all systems; generally, a two-stage drying program is used, beginning typically at 100 to 150 °C for 10 to 15 seconds, followed by a higher temperature at 150 to 250 °C for 10 to 30 seconds.

7.2.2.3 Pyrolysis

The pyrolysis, or char, stage is the thermal pretreatment step designed to remove as much matrix (blood, urine) as possible without volatilizing the analyte. Addition of a phosphate modifier stabilizes lead to temperatures of up to 900 °C. Without a modifier, Pb would be lost below 600 °C. A simple pyrolysis study should be conducted by varying the pyrolysis temperature and recording the integrated absorbances (A_i) for both blood and aqueous standards. Use a fixed atomization temperature that is recommended for lead. Plot the data as $A_i(y)$ versus indicated temperature (T). Use a pyrolysis temperature about 100 °C below the temperature where Pb is lost.

7.2.2.4 Atomization

A similar study should also be conducted to optimize the atomization temperature. The atomization hold time should be sufficient to ensure that the peak has reached its maximum absorbance and returned to baseline. There may be a small difference between the appearance time for blood and standards, but this is irrelevant if the integration window is set wide enough to capture both signals accurately. The atomization profile is a good diagnostic tool and should be displayed if possible after the run is complete. The peak shape should be nearly symmetrical and without shoulders. The peak width should be 0.2 to 1.0 second at half-height. (Figure 1 shows a typical atomization peak.)

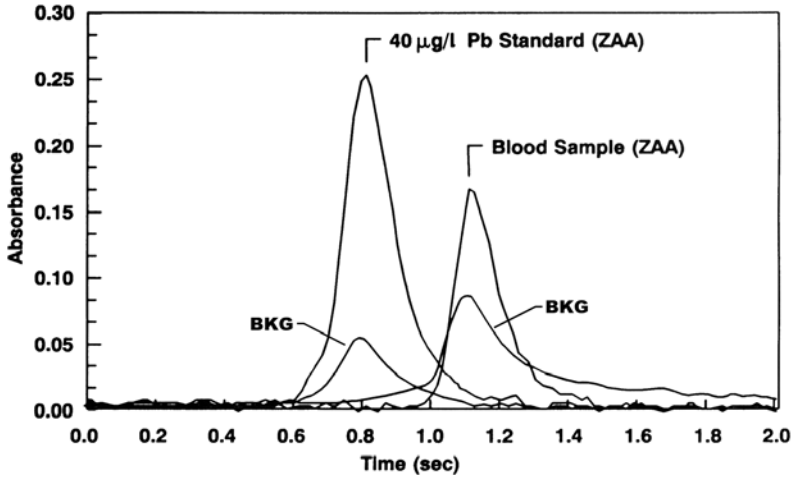

Figure 1. Typical Background (BKG) and Zeeman Atomic Absorption (ZAA) Profiles for Pb Standards and Blood Samples Using a Graphite Furnace Atomic Absorption Spectrophotometer. From *Spectrochim. Acta.* Vol 48B; No. 6/7. Parsons PJ, Slavin W. A rapid Zeeman graphite furnace atomic absorption spectrometric method for the determination of lead in blood. 1993:925. Reprinted with permission from Elsevier Science.

7.2.2.5 Cleaning

The tube-cleaning stage should be hot enough to ensure that the matrix is removed and no analyte carryover exists.

7.2.3 General Requirements

(1) Fixed or adjustable micropipettes that can deliver 50 µL, 200 µL, 1.0 mL, 2.0 mL, and 5 mL. Alternatively, an automatic pipet device equipped with a 2,000-µL dispensing syringe. The device should also have a 2,000-µL and a 200-µL sampling syringe.

(2) Volumetric glassware (Class A): five 100-mL; 1-, 3-, 6-, and 10-mL pipets. All glassware must be acid-washed in 10% (v/v) HNO_3 before use. Note that 10% (v/v) nitric acid is prepared relative to concentrated HNO_3, i.e., 10 mL concentrated HNO_3 per 100 mL H_2O.

(3) Graduated borosilicate glass cylinders: 50-, 100-, and 1,000- mL sizes, acid-washed.

(4) Five 125-mL, acid-washed plastic bottles for storing intermediate and working standards.

(5) One 500-mL acid-washed plastic bottle for storing modifier diluent.

(6) Biosafety cabinet for preparing blood/urine specimens (recommended but not required).

7.3 Reagents and Other Materials

(1) Lead nitrate primary standard solution (1,000 mg/L), certified and traceable to the National Institute of Standards and Technology (NIST, Gaithersburg, MD) or equivalent (see Section 9.2.1).

(2) Concentrated nitric acid (HNO_3), trace-element grade or ultra-high-purity grade.

(3) Deionized water suitable for trace-element work (i.e., NCCLS Type 1 water with a resistivity of >10 MΩ · cm).

(4) Triton X-100®, nonionic detergent, high-purity grade.

(5) Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) modifier. Use a grade (e.g., HPLC grade) that is consistently free from significant lead contamination, i.e., integrated absorbance of the blank solution is <0.005 second.

(6) Blood-based quality control materials, including a low-level (<10 µg/dL), an intermediate-level (20 to 30 µg/dL), and a high-level (40 to 60 µg/dL) material.

(7) NIST (National Institute of Standards and Technology) SRM 955b Lead in Blood (four levels) or equivalent. These should be run periodically to verify accuracy and/or troubleshoot an unexplained bias (see Section 9.1).

(8) Disposable, noncolored plastic micropipette tips, 1- to 100-µL and 1- to 1,000-µL sizes.

(9) Disposable autosampler cups, either 2-mL (standards) or 0.5-mL (samples), screened for lead contamination. The use of 0.5-mL cups is preferred for samples, because the total volume of diluted blood is only 0.5 mL (50 µL + 450 µL modifier), and optimizing the autosampler tip depth is less critical.

(10) Laboratory tissues/wipes.

7.4 Preparation of Reagents, Standards, and Specimens for Analysis

7.4.1 Modifier Preparation (0.2% (v/v) HNO_3, 0.5% (v/v) Triton X-100®, 0.2% (w/v) $NH_4H_2PO_4$[b])

(1) **Stock solution 10% (v/v) Triton X-100®:** Using a volumetric pipette, transfer 10 mL of Triton X-100® to approximately 80 mL of deionized water, warmed slightly in a glass beaker. Mix thoroughly using a magnetic stir bar for at least one hour. When cool, transfer to a 100-mL volumetric flask, and dilute to the mark. Prepare monthly and store at ambient temperature, appropriately labeled with date and analyst's initials.

(2) **Stock solution 20% (w/v) $NH_4H_2PO_4$:** Dissolve 20 g $NH_4H_2PO_4$ in approximately 75 mL of deionized water in a glass beaker, dilute to 100 mL in a volumetric flask. Prepare every six months and store at 2 to 8 °C, appropriately labeled.

(3) **Modifier diluent:** Add approximately 300 mL of deionized water to a 500-mL flask. Using a micropipette, carefully add 1.00 mL of concentrated HNO_3 and swirl to mix. Add 25 mL of 10% stock Triton X-100®, 5 mL of 20% stock $NH_4H_2PO_4$, and dilute to 500 mL with deionized water. Mix thoroughly and transfer modifier diluent to a 500-mL opaque plastic bottle labeled with contents, concentrations, and date of preparation. Store at room temperature and prepare monthly.

7.4.2 Preparation of Aqueous Pb Calibration Standards

Three working Pb standards are prepared by serial dilution of a 1,000-mg/L primary Pb standard, traceable to an appropriate reference material. The intermediate stock solutions and working standards are all maintained at 1% (v/v) nitric acid relative to concentrated HNO_3, i.e., 1 mL concentrated HNO_3 per 100 mL standard.

Pb Stock Solution: 10 mg/L Pb

Pipet 10 mL of the 1,000-mg/L Pb stock into a 1-L volumetric flask. Very carefully add 10 mL of concentrated HNO_3 and dilute with deionized water to yield 10 mg Pb/L in 1% nitric acid. Observe stringent safety precautions while performing this procedure, i.e., gloves and eye protection. Prepare monthly and store at room temperature.

Pb Intermediate Standard Solutions

Pipet aliquots of the Pb stock solution, as indicated in the table below, into acid-washed 100-mL volumetric flasks. Dilute to 100 mL with 1% HNO_3 to yield intermediate standard solutions.

Pipet from 10 mg/L Pb stock solution	Dilute with 1% HNO_3 to:	intermediate Pb concentration
1 mL	100 mL	100 µg/L (10 µg/dL)
3 mL	100 mL	300 µg/L (30 µg/dL)
6 mL	100 mL	600 µg/L (60 µg/dL)

[b] Given a modifier concentration in diluted blood of 0.18% w/v, the total mass of $NH_4H_2PO_4$ deposited on the platform using a 12-μL injection volume is 21.6 μg.

Transfer each intermediate standard solution to an acid-washed 125-mL plastic bottle, appropriately labeled. Prepare these solutions monthly.

Working Calibration Standards (S_0 to S_3):

Prepare the daily working standards directly in 2-mL furnace autosampler vials by diluting the intermediate standards with modifier stock solution by a factor of 10, i.e., pipet 100 µL of each intermediate standard and 900 µL of modifier into three 2-mL vials (S_1 to S_3). Fill another vial with 900 µL of modifier and 100 µL of deionized water to serve as the calibration blank (S_0).

7.4.3 Preparation and Use of Matrix-Matched Pb Calibration Standards (Alternative)

While it is generally simpler to use aqueous Pb standards for calibration purposes, this may prove difficult or even impossible with some furnace instrumentation, particularly older equipment available prior to the mid-1980s. In such cases, it may be necessary to use matrix-matched standards for calibrating the instrument for the BPb determination. The analyst will require access to a pool of blood that is known to have a nondetectable lead concentration (<1 µg/dL). It is more convenient to use an animal blood pool for this purpose. If the analysis is for lead in urine, then the matrix for matching ought also to be urine. The analyst will require access to a pool of urine that is known to have a nondetectable lead concentration (i.e., less than the method detection limit).

The preparation of matrix-matched working calibration standards involves the method of standard additions to aliquots of a single pool of blood (urine) with a nondetectable lead concentration. This ensures that the compositions of the working calibration standards and of the diluted blood (urine) specimens differ only in their lead concentrations. Thus any interference effects on analytical sensitivity from the sample matrix are eliminated.

A reagent blank (S_r) is prepared without any blood (urine) but which in all other aspects has a composition identical to that of the lowest working calibration standard (S_0-zero addition). The lead signal obtained with this blank is subtracted from all working calibration standards and from all diluted blood (urine) specimens to correct for adventitious lead concentrations in reagents and for any artefactual instrumental signals.

Most instruments have software packages which automatically use reagent-blank-subtracted signals to construct “matrix-matched” or “additions” — calibration curves, in which the lead concentration in the lowest working standard (S_0) is computed from the intercept at zero addition. The blank-subtracted lead concentrations in all diluted blood (urine) specimens are then calculated from the slope of the calibration curve.

The stock and intermediate standard solutions are prepared in the same manner as for the aqueous Pb standards (see Section 7.4.2).

Three working Pb standards are prepared by serial dilution of a 1,000-mg/L primary Pb standard, traceable to NIST or equivalent. The intermediate stock solutions, and working standards, are all maintained at 1% (v/v) nitric acid relative to concentrated HNO_3.

Working Calibration Standards (S_0 to S_3):

The daily working standards are prepared directly in 2-mL furnace autosampler vials by diluting the intermediate standards with modifier stock solution by a factor of 10, and adding during this step, an aliquot of “blank” blood to match the matrix of the samples, i.e., pipet 100 µL of each intermediate standard +100 µL of blank blood, +800 µL of modifier into four 2-mL vials (S_0 to S_3). Note the intermediate standard for S_0 will simply be 100 µL of 1% HNO_3. Fill another vial with 800 µL of

modifier and 200 µL of deionized water to serve as the reagent blank (S_r). If the analysis is for lead in urine, then the matrix added to the standards should be urine.

7.4.4 Blood Specimens and Quality Control Materials

Although the procedure described below is specific to a manual micropipetting device, an autopipetter/dilutor may be substituted with appropriate modifications to the procedure.

(1) Load the furnace autosampler carousel with an appropriate number of vials. For aqueous calibration, dispense 450 µL of modifier into each one. If using matrix-matched calibration, dispense 400 µL modifier and 50 µL of deionized water into each one.

(2) Ensure that capillary whole blood (red cells and plasma) specimens are thoroughly mixed by inverting or vortexing microtubes before removing a sample for analysis. Care should be taken to ensure that microtube caps are firmly closed before attempting to vortex. Venous blood tubes should be placed on a laboratory rocker or rotator for at least five minutes prior to pipetting to ensure thorough mixing. In some microtubes, there may be a tendency for red cells to accumulate in the capillary probe. Ensure that these red cells are dislodged before sampling.

(3) Carefully remove the tube cap using a laboratory wipe to prevent contaminating the glove with blood, and withdraw a 50-µL aliquot. Excess blood adhering to the outside of the pipet tip should be removed with a laboratory wipe using a gentle rolling action. Avoid touching the tip end. Transfer the 50-µL aliquot into the autosampler vial. With manual pipets, pumping several times ensures that the red cells are lysed and the blood sample is thoroughly mixed with the modifier. Note the autosampler position and record the blood ID number.

(4) If lyophilized (freeze-dried) blood-control materials are used, they should be reconstituted according to the manufacturer's instructions. It should be noted that reconstituted freeze-dried blood is a suspension rather than a solution, and must be mixed well to obtain reliable results. If reconstituted freeze-dried blood is mixed too vigorously, bubbles will result leading to pipetting errors. It is recommended that freeze-dried blood be reconstituted and then mixed by mechanical rotation for at least 20 minutes before removing an aliquot for analysis. Subsequently, the materials should always be placed on a laboratory rocker prior to removing aliquots for analysis to ensure that no settling occurs. The mixing should result in a material that has no bubbles or visible sediment. Prepare three QC materials of low, intermediate, and high concentration for each carousel run. All QC materials should be prepared by the analyst preparing the clinical specimens.

(5) To extend the useful life of controls, larger quantities of well-mixed control can be aliquotted into smaller acid-washed cryovials and frozen for later use. A fresh control should be prepared at least weekly, unless longer stability can be demonstrated.

7.4.5 Urine Specimens and Quality Control Materials

(1) Handle the urine specimens in the same way as blood specimens for lead determination, but use urine-specific QC materials.

(2) Ensure that each urine specimen is thoroughly mixed before removing a 50-µL aliquot.

(3) Remove a 50-µL aliquot of urine and transfer to the vial, pumping to ensure good mixing.

(4) Note the autosampler position and record the urine ID number.

7.5 Furnace Operation and Maintenance

7.5.1 Graphite Tubes, Platforms, and Contacts

(1) Record the number of firings performed with each graphite tube. The analyst should establish normal tube lifetimes for a specific furnace system in terms of number of tube firings. If this value is exceeded, the tube-and-platform combination should be changed, as it is unwise to push the tube to the point of failure. Tube failure may invalidate some analytical results in that run, and may damage the graphite contact rings.

(2) Periodically (e.g., daily) remove the graphite tube/platform, and inspect the condition. Discard the tube/platform if it is pitted or it contains noticeable deposits.

(3) Clean the furnace before inserting a new graphite tube. A cotton-tipped stick may also be used to clean graphite contacts. Stubborn deposits may be removed with 1% (v/v) HNO_3. Worn or pitted contacts may result in skewed absorbance profiles and poor precision.

7.5.2 Autosampler Alignment

(1) With a flashlight or other local light source illuminating the furnace opening, check that the autosampler tip enters the graphite tube without touching the wall.

(2) Use a small mirror (e.g., a dental mirror) to view the tip position in the furnace and adjust until the tip is 0.5 to 1 mm above the platform.

(3) Check for proper alignment of the autosampler by observing sample deposition on the platform. ***This is critical for unattended operation***. The tip must deposit the entire sample onto the platform. If the tip is too high in the tube, the sample will creep up the tip and deposit onto the wall. Do not continue with the analysis until the sample is deposited onto the platform and *only* onto the platform.

7.5.3 Sample Drying

Once the alignment is correct, check for proper drying. The sample must dry without boiling or sputtering. This is especially important when a new tube or contact rings are installed. Check the drying cycle with blank modifier first, and then with a diluted blood sample. If sample sputters while drying, *decrease* the drying temperature by 10 °C and recheck. *Optimum drying parameters are critical for accurate and precise operation*. Do not continue until the drying is optimized.

7.5.4 Instrument Calibration, Calibration Verification, and Control

(See Section 9.2 for a more detailed discussion of laboratory QC procedures.)

(1) Run the calibration cycle for the blank and each standard. If the integrated absorbance of the blank is >0.005 seconds, stop the run and investigate the source of contamination.

(2) When the calibration is finished, note the correlation coefficient of the regression fit. If the correlation coefficient is <0.995, or if the characteristic mass (see Section 7.7.2) is outside the established range for the equipment, investigate the problem before proceeding. Proceed with QC samples to ensure that the calibration has been verified.

(3) When the analytical run is complete, record the results of each QC material for each instrument (mean ±SD or range) on a laboratory control chart. Record the slope of the calibration line and

the lamp energy in a routine maintenance book. These data are useful for troubleshooting if quality control problems arise. See Section 7.7.

7.5.5 Number of Replicate Injections

The availability of autosamplers for depositing analytical samples on the platform in furnace AAS has greatly improved within-run precision. Thus, the number of replicate injections used will depend on the within-run precision attainable. Some analysts insist on using more than one injection within-run for calibration standards and test samples. Replicate injections are clearly recommended if the within-run precision is worse than ±10% (RSD), and may even be desirable in situations where the routine within-run precision is better than ±10% but where the autosampler may occasionally refuse to deposit the sample and proceeds to the next. The analyst should use good judgment in this respect, but clearly the number of replicate injections selected should be the same for calibration standards, test samples, and quality control materials.

7.6 Calculations

7.6.1 Reporting Blood Lead Results and Linearity (see Section 9.2.2)

Modern furnace instruments are now almost always controlled by software that can perform the calibration calculations, and report results in concentration units, including converting them from µg/L to µg/dL, and multiplying by a fixed dilution factor. Blood lead results >60 µg/dL, or greater than the highest standard, should be diluted into the linear calibration range. Although current furnace autosampler systems can perform dilutions on-line, this may not be reliable. Autosampler dilution procedures should be thoroughly validated against a manual dilution procedure. Reject any analytical results that have not been validated as part of a within-control group and repeat the analysis.

7.6.2 Significant Figures

The number of significant figures to which BPb results are routinely reported is indicative of the routine precision of the analytical method. The convention in analytical chemistry is to report all digits that are certain, plus the first uncertain one.[146] Most routine BPb methods developed prior to the 1980s were only capable of ±1 to 2 µg/dL at 10 µg/dL or worse precision. Thus, routine BPb results should be rounded to the nearest integer for clinical purposes. Since the definition of lead poisoning in the U.S. has been lowered from 25 µg/dL to 10 µg/dL BPb, analytical methods with improved precision have been developed for determining the lower concentrations of lead in blood. For example, current furnace AAS methods with Zeeman background correction can attain a precision (between-run) of approximately ±0.2 µg/dL at low levels.[74,147] With repeated analyses (n>3), therefore, blood level results can be reported to the first decimal at low levels with some confidence. Of course, each laboratory should validate within-run and between-run precision for a routine BPb method to justify reporting results with such implied precision, but for many routine BPb methods in use today, such precision is not justified for a single analysis, and the clinical value is open to misinterpretation. Therefore, routine BPb results should be rounded to the nearest whole number. This would be equivalent to reporting BPb results to ±0.01 µmol/L.

7.6.3 Precision

Method precision is defined in terms of the sample standard deviation:

$$s = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{n - 1}}$$

Evaluation of precision should be reported for both within-day and day-to-day.

Precision may also be defined as the relative standard deviation (RSD), or coefficient of variation (CV), and expressed:

$$RSD = \frac{S_{n-1}}{mean} \bullet 100$$

For a detailed precision protocol, refer to the most current edition of NCCLS document EP5—*Evaluation of Precision Performance of Clinical Chemistry Devices*.

7.6.4 Characteristic Mass (m_0)

The characteristic mass, (m_0) is defined as the mass of analyte (in pg) required to produce an integrated absorbance of 0.0044 seconds, and can be calculated using the blank-corrected, integrated absorbance value (A_i) for a standard of known concentration (µg/L) and the volume injected (µL) into the furnace using the following formula:

$$characteristic\ mass,\ m_{0,}\ (pg) = \frac{conc.\ (\mu g/L) \bullet vol.\ (\mu L) \bullet 0.0044\ s}{integrated\ absorbance,\ A_i\ (s)}$$

The "expected" Pb characteristic mass value for the furnace AAS instrument used may be obtained from the manufacturer's technical manuals and documentation or from the manufacturers technical support staff. However, as the characteristic mass is dependent upon a number of variables, including accuracy of the autosampler pumps and instrument electronics and, in some cases, furnace heating rate, it is important that the "experimental" characteristic mass value be obtained for each instrument used for blood Pb determinations.

The "experimental" characteristic mass value may be established by determining the mean characteristic mass value obtained over a period of two to four weeks. If the lamps, graphite tubes, platforms, and graphite electrodes are purchased from the instrument manufacturer, the calculated "experimental" characteristic mass value should be within ±20% of the manufacturer's "expected" value; if it is not, consider the use of another source of standards. If the inconsistency cannot be resolved, contact the instrument manufacturer for advice.

It should be noted, however, that failure to meet the instrument manufacturer's "expected" characteristic mass value is not necessarily an indication of a problem, especially if the analytical results for the proficiency and quality control materials are within the acceptable range.

Once the "experimental" characteristic mass is obtained for each instrument, the characteristic mass should be monitored on a daily basis. Under normal conditions, the characteristic mass should be reproducible within ±20% of the "experimental" value on a daily basis. If the characteristic mass value falls outside of these limits, refer to Section 7.7.

7.6.5 Detection Limit (DL)

The **detection limit (DL)** is a function of two analytical parameters: **sensitivity** and **precision**. In furnace AAS, the instrumental DL is defined as the concentration equivalent to three times the standard deviation of the blank measurement. To calculate the method-specific DL, a diluted blood specimen with a lead concentration close to the instrumental DL is analyzed at least ten times, and the sample standard deviation calculated. If the BPb method precision is ±0.3 µg/dL near the DL, the method DL would be 0.9 µg/dL. Most analysts would round this value to 1 µg/dL for routine reporting purposes. Some analysts also calculate the **lowest determinable concentration (LDC)**, above which the concentration can be reported with more confidence. There is no generally accepted definition of this parameter; some use five

times the SD, some ten times the SD, and others specify a precision of around 5% RSD. The method DL cannot be better than the instrumental DL.

7.7 Troubleshooting

Analytical problems with this analysis usually appear with internal QC materials or external proficiency materials that are out of the acceptable range. If the method and instrumentation used have previously proven capable of obtaining accurate results, then the problem most often lies in the standards, the instrumentation, the blank, or the internal QC material, rather than in the developed method.

The following guideline may be useful in determining the source of the problem with QC results; however, it may also be used for troubleshooting problems with the characteristic mass (see Section 7.6.4).

7.7.1 Internal QC Results Show an Unacceptably High Bias

- **If characteristic mass is higher than the "experimental" value by more than 20%, (indicative of poorer than usual sensitivity), then:**

(1) Check integrated signal for blank, if >0.005 seconds, rerun standard using a new, acid-washed cup.

(2) Verify all instrument parameters including slit width, lamp current, furnace time, temperatures, and gas flows.

(3) Verify correct addition and preparation of matrix modifier.

(4) Use a small mirror to check for proper pipetting and smooth, complete drying prior to the pyrolysis step.

(5) Prepare fresh modifier solution and redilute stock and intermediate standards.

(6) Change the tube and platform.

(7) Check lamp energy. Try another lamp if possible.

(8) Prepare standard from an independent source.

(9) Change graphite contacts (electrodes).

If none of these steps succeed in elucidating the source of the problem, it may be beneficial to determine the characteristic mass of an analyte other than Pb to check whether there is a systematic hardware problem. If there are problems with gas flows or dispensing the correct sample aliquot, these will be evident (in varying degrees) in the determination of other elements.

If the analyst is unable to establish the source of the problem, the technical support personnel of the instrument manufacturer should be contacted.

- **If the characteristic mass is within ±20% of the "experimental" value, then:**

(1) Prepare a new diluted aliquot of the QC material using a new acid-washed cup.

(2) Verify that the QC lot or batch number has not changed.

(3) Analyze a Standard Reference Material (e.g., NIST 955b).

7.7.2 QC Results Show an Unacceptably Low Bias

- **If the characteristic mass is more than 20% lower than the "experimental" value (indicative of better than expected sensitivity), then:**

(1) Verify all instrument parameters including slit width, lamp current, furnace time, temperatures, and gas flows.

(2) Use a small mirror to check for proper pipetting and smooth, complete drying prior to the pyrolysis step.

(3) Redilute stock and intermediate standards.

(4) Check for possible sources of contamination, including sample cups and pipette tips.

(5) Change the tube and platform.

(6) Check lamp energy. Try another lamp if possible.

(7) Prepare standard from an independent source.

- **If the characteristic mass is within ±20% of the "experimental" value, then:**

(1) Prepare and rerun a new diluted aliquot of the QC material.

(2) Verify that the QC lot or batch number has not changed.

(3) Analyze a Standard Reference Material (e.g., NIST 955b).

7.7.3 Visual Examination

While the above troubleshooting procedures provide some guidance for solving problems related to QC material, another good tool for troubleshooting is the visual examination of the Pb peak profiles. An experienced analyst can quickly ascertain the existence of poor drying conditions by the presence of multiple or "atypical" background or analyte peaks. A shift in the peak profile, indicating earlier analyte volatilization, may indicate an incorrect atomization temperature or ineffective delay in atomization from the platform (i.e., a tube/platform problem). Broadening of peaks may indicate salt condensation at the interface of the tube and contacts (electrodes) or worn contacts.

For this reason, it is advisable to store some typical "normal" peak profiles as well as some "problem" situations profiles (such as in improper pipetting, drying, etc.). Peak profiles may be stored on the computer for later retrieval or in printed form in a notebook or similar storage device. As disk capacity may be a problem for some systems, it is suggested that each laboratory establish a scheme for archiving peak profiles.

8 Procedures for Pb in Blood by Anodic Stripping Voltammetry (ASV)

8.1 Method Principle

Anodic stripping voltammetry is an electroanalytical technique based on polarography. In this application, an electrode assembly is used to reduce (plate) the electroactive analyte(s) onto a mercury film, after which the amalgamated reduced species are oxidized (stripped) and the resulting charge flow (area under the current-time curve) measured and related to concentration.[148,149] Lead can be reversibly oxidized and reduced according to the equations in the following figures:

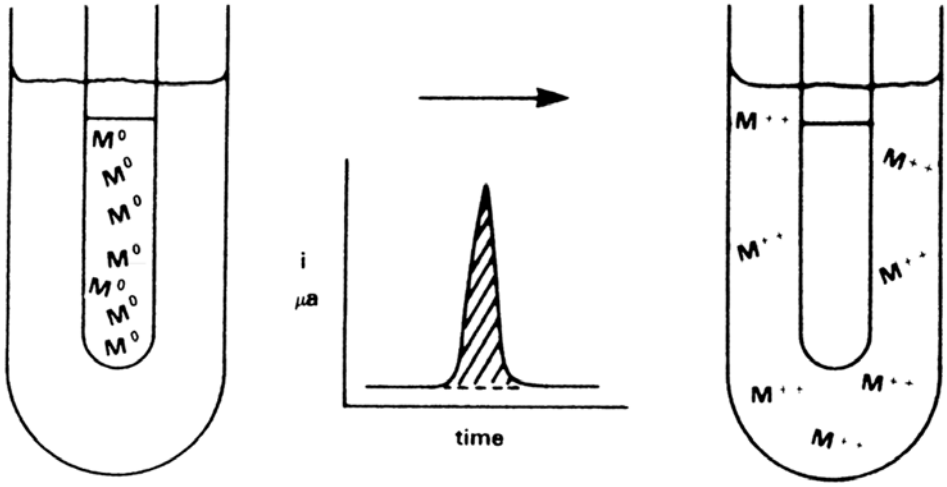

Figure 2. **Reduction/Amalgamation. NOTE:** $Pb^{2+} + 2e^{-} + Hg(m) = Pb(Hg)$ = amalgamated lead.

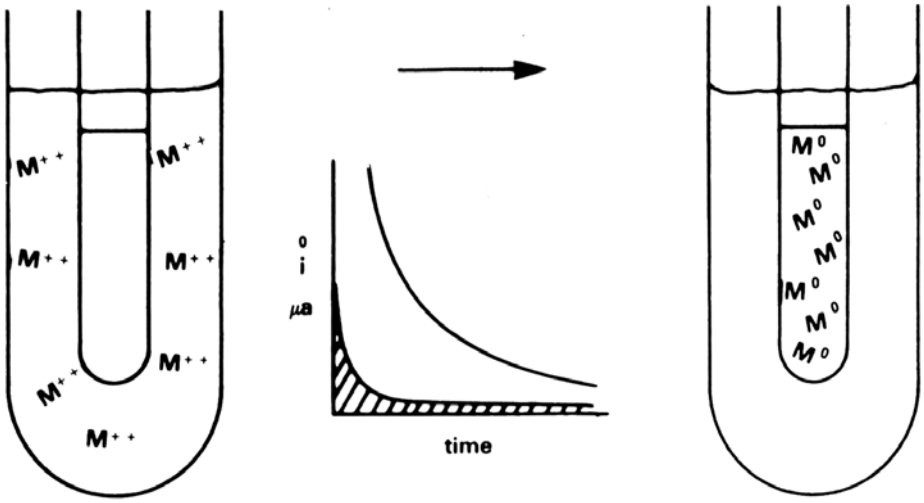

Figure 3. **Oxidation. NOTE:** $Pb(Hg) = Pb^{2+} + 2e^{-} + Hg(m)$.

The concentration of lead is related to the total charge, measured as the area under the current-time curve. Under the conditions employed for determination of lead in the most widely used commercial systems, the oxidation step (Figure 3) is performed with a staircase square-wave form, in order to reduce the effects of "charging" current due to the capacitance of the electrical double layer next to the mercury electrode. The combination of the preconcentration step (by amalgamation—Figure 2) and the avoidance of capacitive ("charging") current results in a measurement system with high sensitivity.

8.2 Apparatus

8.2.1 Instrumentation

Anodic stripping voltammetry may be performed with any electronic control device and electrode assembly. In this section, a commercially available ASV instrument for blood lead is used.[150] The electrode assembly includes the test or "working" electrode, which may be carbon, with a thin-film mercury surface, a mixing device, a reference electrode (e.g., silver-silver chloride or saturated calomel

electrode), and a counter electrode (e.g., platinum or platinum alloy) to carry most of the current. The electronic unit is used to control the potential(s) of the electrodes, initiate and complete the plating cycle (equations above), integrate the current-time curve, and calculate the analytical results—often directly in µg/dL (BPb).[151]

8.2.2 Sample Preparation

Blood samples must be pretreated prior to analysis to convert lead to the free Pb^{2+} state for subsequent plating onto the working electrode. This may be accomplished either by digestion in mineral acid(s) or by incubation in a decomplexing reagent. The most common approach currently utilizes incubation with a decomplexing reagent, and is the approach recommended by the manufacturer of the most widely used commercial ASV instrument for BPb.[151]

This reagent is used to decomplex the lead from the erythrocytes or complexing agents (e.g., EDTA). A slight excess of Hg^{2+} is included to maintain the coating of the mercury electrode within its broad range of functionality. Several recipes exist for preparing the decomplexation reagent, one of which contains the following ingredients: chromium chloride hexahydrate, calcium acetate monohydrate, mercuric ion, and Triton X-100® (surfactant).[151] An alternative formulation which has been used successfully for many years[150] is made up of the comparable components. Only laboratories experienced in electrochemical analyses should attempt the preparation of their own decomplexation reagent for use in BPb determinations. These reagents should be prepared with high-purity components to minimize lead contamination to levels below the method detection limit.

Recommended sample incubation times in decomplexing reagents vary from as little as 45 minutes to 18 hours (overnight). Currently there is no definitive data on the minimum decomplexation times. Some laboratories using commercially prepared decomplexation reagents recommend overnight incubation.[152] Other laboratories have reported minimum incubation times of less than one hour using alternative recipes.[150]

If an EDTA collection tube is received with less than half the recommended fill volume, i.e., the EDTA concentration is >1.5 mg/mL, this may result in a low bias with ASV. Problems with ASV have been reported when analyzing blood samples drawn from subjects who are currently undergoing treatment for lead poisoning with the chelation agent Succimer (DMSA). For DMSA-containing blood samples, ASV is not recommended (Jones RL, personal communication, 1997).

8.2.3 Calibration

Calibrators can be purchased from commercial sources or can be prepared by the analyst from well-characterized whole blood, both of which should be traceable to NIST or equivalent (refer to Section 9.1).

While it is good laboratory practice to calibrate analytical instrumentation for inorganic analytes using at least three standards and a blank, there are situations in which a two-point calibration is sufficient. Such situations are only possible where the analytical response is linear.

In situations where a two-point calibration is possible, a stringent quality control protocol is recommended. Refer to Section 9.2.

8.2.4 Instrument Setup and Maintenance

Users of commercial ASV instrumentation specifically for BPb determinations should follow the manufacturer's recommendations for instrument operation and electrode maintenance.

Note that high-concentration samples may result in memory or carry-over effects; therefore running a blank sample following a high sample is desirable.

The reference electrode assembly should be maintained according to the manufacturer's instructions.

It may be necessary to run two or more commercial controls to verify the calibration curve. If it is not within limits (about ±10 %), the instrument should be recalibrated.

Copper can interfere with the blood lead determination by ASV. When lead and copper strip from mercury amalgams into a supporting electrolyte to the +2 oxidation state, the lead and copper peaks are well resolved whether one uses staircase or differential pulse techniques. However when the supporting electrolyte has a high enough chloride ion concentration, copper will strip to the +1 oxidation state, and the resulting peak will be much broader. Intermediate chloride ion concentrations can result in a mixture of +2 and +1 states and, again, the overall envelope of the "copper peak" is both broader than normal and decidedly skewed. All of this can result in loss of peak resolution to the point where elevated copper concentrations will begin to produce false-negative blood lead results.[152] Elevated serum copper concentrations are normally encountered in the third trimester of pregnancy, in cases of Wilson's disease, and in women on some birth control formulations. It may also occur under some conditions extant during chelation therapy. For instance, penicillamine therapy may cause elevated serum copper levels—at least initially. This interference can be minimized by careful selection of the integration set point—the potential at which current-time area integration is initiated.

8.2.5 Reporting Blood Lead Results and Linearity

Refer to Section 7.6 for a discussion on significant figures for reporting blood lead results (7.6.2); precision (7.6.3); and how to calculate a detection limit (7.6.5).

Commercial ASV instrumentation is reported to be linear up to 100 µg/dL. However, there may be some situations where BPb test results are greater than the highest calibrator used to calibrate the instrumentation. In these situations, it is normal laboratory practice to dilute "over-range" test samples so that the Pb concentration falls within the calibration. Since it may not be possible to dilute ASV over-range blood samples owing to matrix effects, a suitable approach is to validate the linear dynamic range using well-characterized reference samples that include the elevated BPb concentration range desired. Alternatively, one can use a linearity check kit provided by the manufacturer. The over-range test sample should be reanalyzed along with an appropriate elevated control sample.

9 Reference Materials and Quality Control for Lead in Blood and Urine

9.1 Reference Materials

Accuracy and comparability have always been important objectives of clinical measurements. Incorrect analyses lead to unsound decisions that can be costly and directly affect the health and well-being of patients, while discrepant results undermine confidence in the diagnostic tests. The incorporation of reference materials into the analytical process is the principal methodology for avoiding such pitfalls and can provide a traceability network as well as a basis for measurement quality assessment.

In an effort to promote greater accuracy in clinical analyses, NCCLS established the National Reference System for the Clinical Laboratory (NRSCL) in 1978. This system defines an accuracy hierarchy for the traceability of clinical measurements in which reference materials play a central role. A reference material is defined by NCCLS as "*a material or substance, one or more of whose property values are sufficiently homogenous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials*." Reference materials connect the base units, moles of substance, with clinical measurements, and this linkage can form the basis of a

traceability network. A certified reference material is a further refinement of a reference material and is defined as "*a reference material that has one or more values certified by a technically valid procedure and is accompanied by, or is traceable to, a certificate or other document that is issued by a certifying body.*" The ISO definition of certified reference material[153] extends this definition by specifying that uncertainties must also be estimated and stated in the certificate.

Certified reference materials have been used as an accuracy base for the production of secondary and internal reference materials. They have also been used as calibrants and have an important role in method as well as instrument development. They are central to quality assurance, because a correct measurement of an analyte in a reference material implies a correct measurement of the analyte in unknowns, provided the reference material meets certain selection criteria. The most important criteria include matrix matching, analyte concentration comparable to the level in the unknown, and a low uncertainty in the certified analyte concentration.

Guidelines for the NRSCL have been published by NCCLS (e.g., NRSCL13—*The Reference System for the Clinical Laboratory: Criteria for Development and Credentialing of Methods and Materials for Harmonization of Results*) and the general schema of their accuracy hierarchy for clinical measurements is laid out in Figure 4.

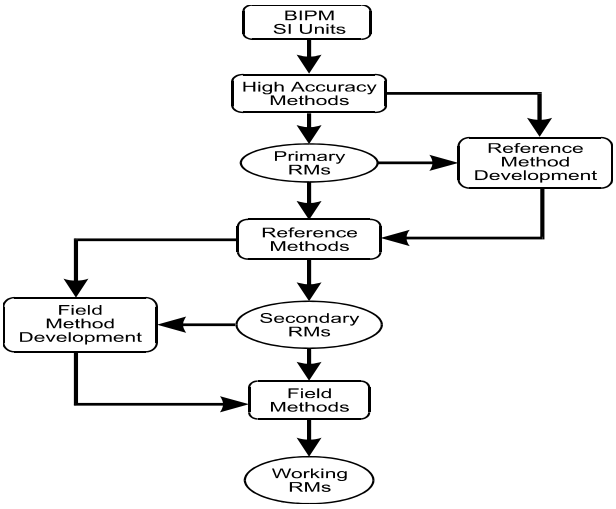

Figure 4. Hierarchy of Reference Materials Providing the Traceability Links for Clinical Measurements. NOTE: At the apex of this system are the fundamental units of measurement, the International System of Units (SI). They are linked via high-accuracy analytical methods to primary reference materials. A primary reference material is[153] "*any certified reference material that is generally accepted or officially recognized as the unique standard for the assay, regardless of its level of purity of analyte content.*" In effect, they are certified reference materials whose values can be accepted without further verification by the user and thus represent an accurate realization of the matrix and analytes being measured. The system then cascades through methods and materials of decreasing accuracy and characterization.

While the use of reference materials is a widely accepted component of quality assurance, what property of the standard is specifically needed to assure blood lead analysis quality? Quality as defined by ISO[153] is "*the totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs.*" While this is a reasonable generalization, it is ambiguous to the extent that stated or implied needs are often poorly known or, in some cases, unknown to the analyst. Comparability, while inspiring confidence, does not necessarily confer any accuracy to a measurement scheme. In fact, strictly speaking, any decision on the quality of a measurement cannot be made by comparability assessments but must rather come from analysts who understand the measurement process in detail and

can therefore quantitatively assess its accuracy. Accuracy, on the other hand, does confer comparability and is therefore the most important component of quality in clinical measurements. Accuracy is a concept that most clinical chemists understand intuitively and scientifically. One of the most direct ways of meeting the needs of accuracy is via a traceability network. Traceability is "*(the) property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons, all having stated uncertainties.*" It is important to note that the degree of accuracy conferred by traceability to value assignments is not specified in the definition but rather that the uncertainties must be correctly carried through the linkages so that the true value of the analyte lies within the estimated bounds.

The NRSCL is specific about the setting of the certification values within their analytical hierarchy. If accuracy is one of the most important features for assuring the quality of clinical measurements, then only high-accuracy methods, where they exist, should be used for the certification process. In the absence of a high-accuracy technique, analyte concentrations could be set by combining the results of at least two independent methods that should agree within the limits of the methods' uncertainties. Within the clinical community, high-accuracy methods are called "definitive methods" and include gravimetric, volumetric, and coulometric analyses, as well as isotope dilution mass spectrometry, all of which can be connected directly to the mole. Definitive methods are characterized as analytical procedures having a valid and well understood theoretical basis as well as methods that yield results that are both precise and that have negligible systematic error.[154,155] The magnitude of the definitive method's final imprecision and bias, expressed in the uncertainty statement, is compatible with the definitive method's stated end purpose. The mean definitive method value is taken as the "true" value. The process of developing and maintaining a definitive method is quite costly and time-consuming and is usually undertaken by national metrological laboratories. Laboratories such as the National Institute of Standards and Technology (United States of America), the Laboratory of the Government Chemist (United Kingdom), the Institute for Reference Materials and Methods (European Union), Bundesanstalt fuer Materialforschung (Germany), National Research Council (Canada), and the National Research Centre for Certified Reference Materials (Peoples Republic of China) have produced certified reference materials as primary standards using definitive methods.

Figure 5 shows how primary and secondary reference-material producers can provide a traceability network. In this example, both Laboratory A and Laboratory B produce field measurements traceable to NIST. For Laboratory A, the traceability arises by the direct use of the Standard Reference Material through Link 1, while Laboratory B uses a secondary reference material (Link 3) that is itself traceable to NIST (Link 2). Because the SRM is certified using a definitive method and the value assignment is based on absolute assessment, the end user has an accurate and direct link with the base units—moles of substance. Within this type of analytical framework, results from Laboratory A will also be comparable with results from Laboratory B. Thus field laboratories can in principle achieve traceability and comparability without direct recourse to the generally more expensive primary reference material produced by national metrological laboratories. If, as in this example, secondary reference material producers are creating traceable reference materials through primary reference materials, the reference materials they provide should enhance rather than degrade the quality of clinical analyses.

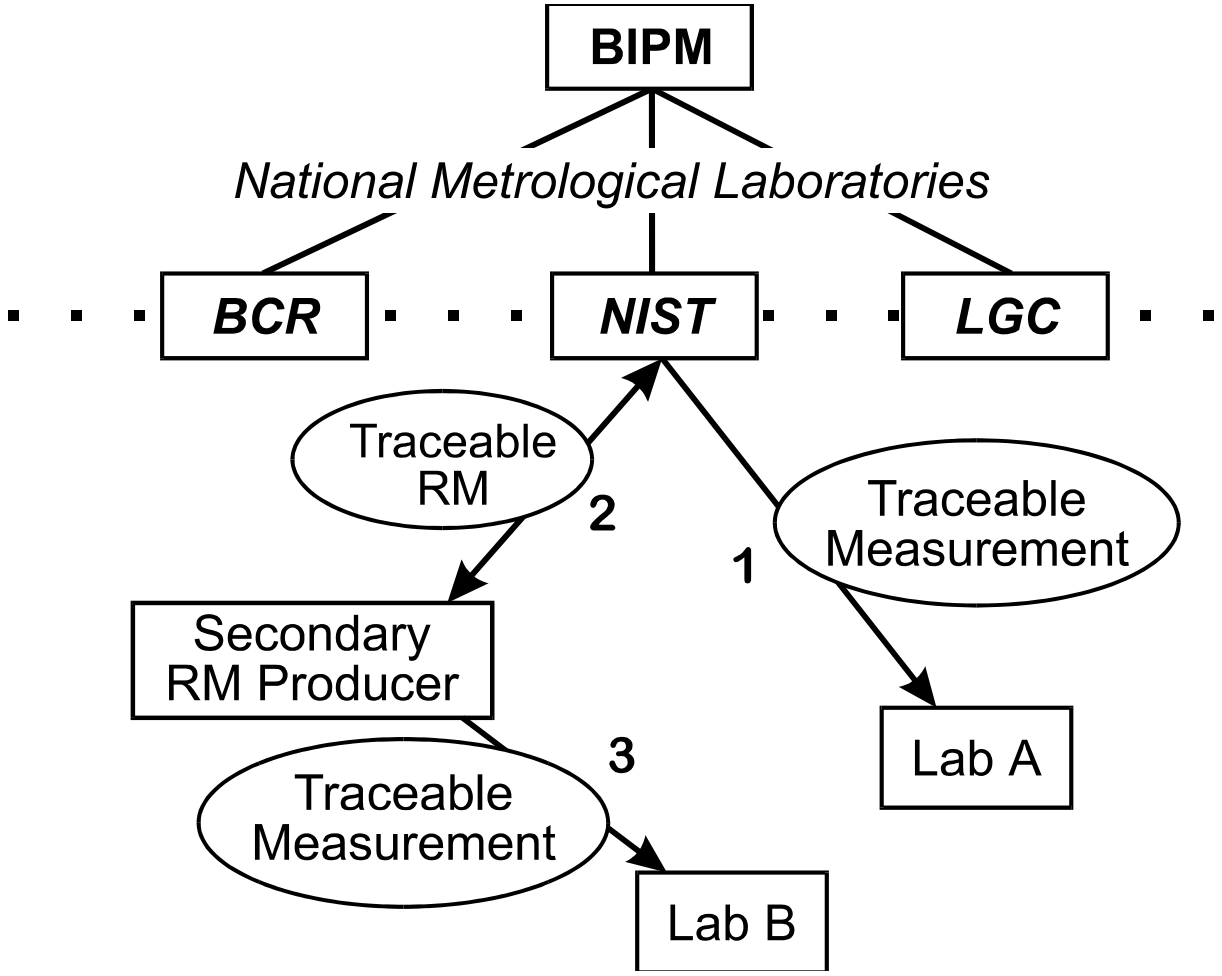

Figure 5. Traceability and Comparability Arising Through the Use of High-Accuracy Reference Materials. NOTE: The abbreviations have the following meanings - BIPM: Bureau of Weights and Measures (International); NIST: National Institute of Standards and Technology (USA); LGC: the Laboratory of the Government Chemist (UK); BCR: Community Bureau of Reference (EU); RM: Reference Material.

Following these guidelines, the ideal certified reference material for blood lead analyses should be a human whole-blood reference material with endogenously bound lead. It should span the range of important analyte concentrations and matrix variations. It should have a certificate containing an accurate realization of the lead concentration together with an uncertainty at a stated level of confidence, all done by a procedure which establishes its traceability to the base SI units. The uncertainty should furthermore be much less than the uncertainty that the application can tolerate and still be useful. Table 2 is a list of blood lead reference materials certified by national and international reference material producers. Information on the type of blood and the form of the lead are listed together with the name of the certifying bodies and the certification method. Even a cursory examination of Table 2 reveals that none of the materials meet all the above criteria for an ideal blood lead reference material. Obviously, compromises have been made due to ethical and other considerations. The blood matrix is generally animal-based rather than human, and the lead is often spiked rather than organically bound. The effect of these variations on the analysis of blood lead has been the subject of considerable research. However, the general consensus is that modern instrumentation is not perceptibly affected by these differences at current levels of precision. The principal area of potentially significant distinction is the certification method. Most of the blood lead materials were certified using nondefinitive-method means or consensus values, with only two sources certifying the lead values by a definitive method—isotope dilution mass spectrometry.

Table 2. Sources of Blood Lead Reference Materials Certified by National and International Reference Material Producers. The certification methods for lead include isotope dilution mass spectrometry (IDMS) and graphite furnace atomic absorption spectrometry (GFAAS). A consensus value assignment is based on pooling the results of a group of laboratories, usually with some statistical filtering of the raw data. A method-mean value assignment involves multiple determinations using a single method in a single laboratory. Independent-method means value assignment results from the averaging of values from two or more independent methods.

Source	Certifying Body	Type of Blood	Levels	Form of Blood	Certification Value
NIST *SRM 955a,b*	National Institute of Standards and Technology	Bovine, endogenous Pb	One SRM, four levels/SRM	Frozen whole blood, 2 mL vials	Absolute Values (IDMS)
NIST *SRM 966*	National Institute of Standards and Technology	Bovine, endogenous Pb, exogenous Cd, MeHg and Hg	One SRM, two levels/SRM	Frozen whole blood, 2 mL vials	Absolute Values (IDMS) and Independent-Method Means
NRCCRM *GBW 09132, 09133, 09134*	Institute for Environmental Health Monitoring	Bovine, exogenous Cd, Pb	Three CRMs, one level/CRM	Frozen whole blood, 3x3 mL vials	Method Means
NRCCRM *GBW 09139/40*	Institute for Environmental Health Monitoring	Bovine, exogenous Cd, Pb	One CRM, two levels/CRM	Freeze-dried whole blood	Method Means
BCR (SM&T) BCR-CRM 194, 195, 196	Institute for Reference Materials and Measurements	Bovine, exogenous Cd, Pb	Three CRMs one level/CRM	Lyophilized whole blood, 5 mL vials	Consensus Values
BLLRS	Centers for Disease Control and Prevention	Bovine, endogenous Pb	Multiple–level RMs	Whole blood, 2 mL vials	Absolute Values (IDMS)
NY State DOH	State of New York Department of Health	Goat, endogenous Pb Bovine, endogenous Pb	Multiple-level RMs	Lyophilized whole blood, 2 mL vials	Consensus Values
CTQ	Centre de Toxicologie du Québec	Human, endogenous Pb	Multiple-level RMs	Frozen whole blood, 2 mL vials	Consensus Values
Referensmaterial AB *AMI B1001, B1002, B1003, B1004, B1005*	Danish National Institute of Occupational Health	Human, endogenous and exogenous Pb, exogenous Cd, Cr, and Co	Five CRMs, one level/CRM	Lyophilized whole blood, 5 mL vials	Method Means (GFAAS)

BCR (SM&T) Institute for Reference Materials and Measurements (Standards Measurement and Testing), Management of Reference Materials (MRM) Unit, Retieseweg, B-2440 Geel BELGIUM
BLLRS Blood Lead Laboratory Reference System, CDC, MS F18, 4770 Buford Highway NE, Atlanta, Georgia 30341-3724 U.S.A.
CTQ Centre de Toxicologie du Québec, 2705 Boulivard Laurier, Sainte-Foy, Québec, CANADA G1V 4G2
NIST National Institute of Standards and Technology, Standard Reference Materials Program, Rm. 204, Bldg. 202, Gaithersburg, Maryland 20899 U.S.A.
NRCCRM National Research Centre for Certified Reference Materials, Office of CRMs, No. 18, Bei San Huan Dong Lu, Hepingjie, 100013 Beijing, CHINA
NY State DOH State of New York Department of Health, Wadsworth Center, P.O. Box 509, Albany, New York 12201-0509 U.S.A.
Referensmaterial AB Referensmaterial AB, P.O. Box 64, S-523 22 Ulricehamm, SWEDEN

A system such as the NRSCL, when properly implemented, will enable all elements of the analytical process to be traceable in an unbroken series of comparisons back to the base unit, the mole. Combining accurately characterized certified reference materials together with suitable and well-defined measurement systems gives the clinical chemist a relatively easy way to ensure that a quantitative transfer of accuracy together with a realistic assessment of uncertainty can occur.

9.2 Quality Control

Quality control (QC) is a broad topic, loosely defined as all the practices used to ensure the accurate generation and reporting of results. A complete discussion of QC is beyond the scope of this document, though many parts are applicable. This section addresses internal QC practices pertaining to calibration, quantitation limits, and the use of control samples in BPb analysis.

9.2.1 Calibration

Establishment of a valid calibration procedure is fundamental to the accuracy of any chemical analysis. Documentation and validation of calibration procedures are recommended.

A minimum of three calibration standards plus a suitable blank is recommended. The standards should cover the commonly encountered range of clinical BPb values, including a standard at or below the threshold of significance (<10 µg/dL), an intermediate value (approximately 30 µg/dL), and a substantially elevated value (approximately 60 µg/dL). Patient specimens with values above this figure are encountered, but they are relatively uncommon. A concentration of 60 µg/dL also coincides with the upper limit of linearity for many instruments in the field.

Calibration standards for atomic absorption methods may be aqueous or blood-based, while electrochemical (ASV) methods without digestion require blood-based standards. Regardless of the material chosen, the standard material must be well characterized, with values traceable to NIST, the Commission of European Communities, or other certifying agency (see Section 9.1).

The frequency with which calibration must be performed should be determined by the laboratory, but should be performed no less frequently than every eight hours of analysis time, unless longer instrument stability has been clearly established.

9.2.2 Quantitation Limits

Distinct from routine calibration procedures, determination of the upper and lower limits of quantitation is also a necessary QC practice. In the simplest case, these limits are defined by the values of the lowest and highest calibration standards, e.g., approximately 10 and 60 µg/dL.

Because medical management decisions may be influenced by the value of the lead result at concentrations exceeding this upper threshold, it is preferable to quantitate such samples using well-validated special analysis procedures. Procedures that can be employed to measure above-range lead concentrations include sample dilution, reduction of sample volume, and the establishment and use of nonlinear calibration curves. In the field, sample dilution is most widely used. Generally, a 1+1 dilution of sample with modifier solution or decomplexing reagent is performed, followed by analysis using standard procedure. A calculation is then necessary to obtain the final result.

Proper validation of this type of procedure is hindered by a lack of suitable reference materials in this concentration range. In the event that the quantitation cannot be verified, results above the highest calibration standard should be reported in alphanumeric form, e.g., >60 µg/dL.

The low-level threshold for reporting quantitative results is often based on the method detection limit (DL) rather than the lowest standard value. This is advantageous, since the method DL is generally significantly smaller than the lowest standard, allowing reliable quantitation of lower BPb concentrations. Information on establishment of the method DL can be found in Section 7.6.5. Results falling below the established method DL (which is often as low as 1 to 2 µg/dL) must be reported alphanumerically, e.g., <1 µg/dL.

Many laboratories define a low-level reporting threshold that is above the method DL, but below the 10 µg/dL interest threshold. This reporting threshold, often 5 µg/dL, is established for convenience, and to reduce possible misinterpretation of small differences in serial results as actual trends in the degree of lead exposure.

Documentation of the method DL and above-range quantitation procedures is recommended.

9.2.3 Quality Control Samples

The daily use of analytical quality control (QC) samples is necessary in order to monitor the daily analytical results. A wide variety of QC materials is available for blood Pb. The compositions of these products vary considerably, and these variations can influence the suitability for QC purposes. The primary characteristic of a suitable QC material is that it be stable and that a large enough quantity be available to satisfy the requirements over the period that the material is stable.

When using commercial control materials, it is important to follow the usage, storage, and handling instructions accompanying the material. Questions about storage or handling of commercial materials should be directed to the material manufacturer.

If using noncommercial QC materials, the laboratory should establish recovery or target values developed over 20 separate runs per level. If aliquotting and freezing the material for extended usage, recovery concentrations should be reproducible.

If commercial QC materials are used, the quantity obtained should be all from the same batch and homogeneous among the several containers supplied. The target value may be assigned within the laboratory by frequent tests on many days using the established blood Pb method under proper control, or by analyzing along with certified reference materials for BPb. Aliquots of this QC material are then stored frozen for use later in the routine analysis with each batch of patient samples in a format discussed below.

Some suppliers provide materials that can be used for QC and that the supplier claims can also be used as a calibrator, or as a certified reference standard (CRM). If it is truly a certified reference material traceable to a primary material, (e.g., SRM material from NIST in the U.S., see Section 9.1), it may be too expensive to use for routine QC. Lyophilized products require careful attention to reconstitution instructions to ensure a homogenous, nonfoamy suspension.

Three concentrations of control material should be used for QC: low<10, medium between 20 and 35, and high >50 µg/dL. There are many valid protocols for the arrangement of controls within an analytical run.

One recommended protocol for control inclusion is as follows:

Position in Run	Number &	Level of Controls
Following calibration	3	1 each level
Every 15 to 20 samples	1	rotate levels
End of run	3	1 each level

Once the target Pb concentration is determined, acceptability limits must be set that reflect the quality of the work in the laboratory. Laboratory regulations within the U.S. require that the acceptability limits be no larger than ±4 µg/dL below 40 µg/dL, or ±10% of the target value above that concentration. However, below 40 µg/dL it is both possible and prudent for the laboratory to set narrower internal limits so that the results are more accurate, especially at the key BPb thresholds of 10 µg/dL and 20 µg/dL. This document recommends that the limits be ±2 µg/dL below 20 µg/dL and ±10% of the target value above that concentration. Since these QC limits must accommodate the imprecision of the method, they should be three times the standard deviation of the method. This implies that the imprecision of the method should be less than 0.7 µg/dL at low concentrations and that the method detection limit should be less than 2 µg/dL. Daily control results should be systematically recorded, and the aggregate data analyzed according to established protocols to evaluate data quality over an extended time period.

10 Laboratory Policy

10.1 Laboratory Certification and Proficiency Testing

Laboratories conducting lead analyses may be required to meet specific regulations. Refer to Appendix B for more detail.

10.2 Follow-up and Rescreening

The CDC have published recommendations for screening children at risk of lead poisoning.[1] Refer to Appendixes C2 and C3 for a summary of these recommendations.

The subcommittee recognizes that two types of reanalyses (i.e., repeat testing and repeat sampling) may be necessary. The circumstances for each are described below.

10.2.1 Repeat Testing

Repeat testing in the laboratory with a new aliquot from the original sample is generally performed by the laboratory to confirm results of the first or second analysis and prior to reporting results.

The following criteria for reanalyzing the specimen are based on retesting the original specimen submitted for the analysis. The primary reason for reanalyzing a specimen is to ensure elevated results were not compromised by bench contamination errors.

(1) Following a single determination, reanalyze the specimen a second time for all results ≥10 µg/dL. In the case of capillary blood specimens, the sample volume may not permit you to reanalyze the specimen.

(2) Report the average of the two results *except* where the difference between result 1 and result 2 is:

- >2 µg/dL for mean BPb values 8 to 20 µg/dL, or
- >3 µg/dL for mean BPb values 21 to 30 µg/dL, or
- >4 µg/dL for mean BPb values 31 to 40 µg/dL, or
- >10% for mean BPb values of 41 to upper limit of calibration.

(3) If any of the above occur, then analyze the specimen a third time. Compare the three results, reject any obvious outlier, and average the remaining two to give a final BPb result.

(4) Dilute and reanalyze all results in duplicate that are initially above the highest standard. For example, the original specimen should be diluted by a factor of 20 rather than 10, (e.g., 50 µL blood + 950 µL modifier for furnace AAS methods). Report the average of the duplicate test results, unless there is a discrepancy of more than 10% between the two. In such cases, the analysis should be repeated again in duplicate.

10.2.2 Repeat Sampling

From a laboratory perspective, repeat sampling, or redraw of a new specimen, is performed as a rescreen or follow-up to results reported as abnormal, borderline, or unsatisfactory for analysis. This is important because the medical and environmental interventions that will be triggered by elevated results should be based on values generated with a high degree of confidence.

Repeat sampling is indicated under the following scenarios:

- Blood lead levels are elevated (>10 µg/dL).
- Initial specimen was unsatisfactory for examination and/or insufficient quantity to perform analysis.
- Laboratory accident occurred that might compromise specimen or data integrity.

The laboratory should recommend drawing a venous specimen for follow-up testing. In general, a venous specimen should be requested for the following:

- Confirmation of an elevated BPb (>10 µg/dL) in a capillary sample.
- Children identified as lead poisoning cases; venous blood is required for the duration of the screening.

10.3 General Policies for Lead Analyses (See Section 6)

10.3.1 Preanalytical Considerations

There are no special instructions, such as fasting or special diets, associated with lead analyses.

10.3.2 Acceptable Specimen (See Section 6)

The preferred specimen for the determination of lead is whole blood with EDTA (nominal concentration 1.5 mg/mL) anticoagulant. EDTA is preferred to heparin, because the latter more frequently leads to microclots. However, heparinized blood may be analyzed if microclots are absent. For urine specimens, no preservative is required, but the specimen must be collected and stored under contamination-free conditions.

Acceptable containers for BPb analyses include venous blood collection tubes, greater than 0.5 mL or plastic microcollection tubes greater than 100 µL in volume; and the containers must be free from significant lead contamination.

At least 500 µL of venous blood should be collected for diagnostic purposes. The minimum blood volume required for a single analysis is 50 µL (0.05 mL); therefore, the minimum capillary blood volume can be as little as 75 µL. It should be noted that this volume is adequate for screening purposes, but not for

diagnostic or medical monitoring purposes. Regardless of the collection container used, it should be filled to the correct collection volume whenever possible.

The use of liquid EDTA tubes can cause a small sample dilution effect in blood lead measurements (Jones RL, personal communication, 1997). Failure to fill the tube to the correct volume increases this effect. Therefore, it is especially important to fill the collection container to volume when liquid EDTA tubes are used. This problem is not observed when dry (powdered) EDTA is used.

10.3.3 Specimen Stability

In general, whole blood specimens may be transported to the laboratory at ambient temperature. If using a private or public delivery service, ensure that the appropriate regulations governing the transport of human clinical specimens are followed. Blood specimens are stable for up to ten weeks if refrigerated at 2 to 8 °C[135] or for up to one year if frozen at -30 to -10 °C. Never freeze blood specimens in glass tubes, since they are likely to crack; transfer into acid-washed cryogenic vials for long-term frozen storage. Specimens may be stored at 2 to 8 °C pending analysis. Portions of the sample that remain after analytical aliquots are withdrawn should be refrigerated at 2 to 8 °C. Samples refrigerated several times are not compromised, unless inadvertent contamination occurs due to improper handling.

10.3.4 Specimen Rejection Criteria

The overall acceptability of a specimen is dependent upon the type of test requested, analytical method in use, previous results, patient history, and risk category. Based on these factors, each laboratory should clearly define criteria for unacceptable specimens, grounds for rejection and actions to be taken when a specimen is deemed unacceptable for analysis. These samples should be reported to the provider in writing and verbally, if possible or deemed necessary, as unacceptable along with a brief explanation of unacceptability. The laboratory should also recommend an appropriate follow-up action.

Guidelines for unacceptable specimens include but are not necessarily limited to:

- Sample received leaking or outer container is contaminated. The laboratory should reserve the right to decontaminate and discard any specimen which may present an unnecessary infectious hazard to personnel.

- Specimen is not properly identified.

- Specimen is inappropriate for the test requested. For example, serum, packed red cells, and plasma received when BPb requested.

- There is insufficient sample quantity to perform the test requested (e.g., <50 µL).

- Specimen is clotted.

- If an EDTA collection tube is received with less than half the recommended fill volume, i.e., “a short draw,” and the proposed analytical method is ASV (excessive EDTA concentrations can result in a low bias with ASV measurements).

Under certain circumstances, a compromised specimen may be considered conditionally acceptable by the laboratory. In these cases the results may be reported to the submitter with a qualifier identifying the precise conditions, reasons, concerns, or situations impacting the degree of confidence, accuracy, and/or validity of the results as reported.

Circumstances under which a specimen can be conditionally analyzed may include but are not necessarily limited to:

- The sample was received incorrectly or inadequately packaged according to the laboratory's written policy (i.e., submitter did not use lead-free collection tubes.[c])
- There is insufficient specimen quantity to perform a confirmation or repeat analysis of an initial or preliminary finding.
- The results can be reported with a reduced degree of confidence that is authorized as acceptable to the healthcare provider or sufficient for the intended purpose of the test.

10.3.5 Contamination

It is important that each lot of specimen collection tubes or storage containers be screened for lead contamination (see Appendix A for details).

10.3.6 Demographic and Analytical Data Integrity Policy

Analytical data must not only be as accurate and precise as scientific technology allows, but must be properly interpreted if it is to be of diagnostic and decision-making value. For this reason, knowledge of patient demographics and case history, specimen demographics, limitations, and uncertainties must be collected and used to define the boundaries in which analytical interpretations can be considered valid. The laboratory should be as concerned about the collection, capture, processing, and storage of demographic and specimen data as they are about the integrity of analytical data, particularly when treatment and intervention measures are at stake. (See Appendix C1 for details on electronic reporting policies in the U.S.)

[c] **NOTE:** Where the concern is that a false-positive result may be reported due to a blood collection tube that is not certified as lead-free, but analysis gives a BPb value that is <10 μg/dL, the result can be reported without qualification. Where BPb results are ≥10 μg/dL, the laboratory report should contain a qualifying statement that use of blood tubes noncertified for the BPb test can produce falsely elevated results. All elevated BPb test reports based on noncertified tubes should recommend repeat sampling prior to initiating chelation therapy or conducting environmental investigations of potential lead sources.

References

[1] Centers for Disease Control (CDC). Preventing lead poisoning in young children: a statement by the Centers for Disease Control. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1991. [Report]

[2] Centers for Disease Control and Prevention. *Screening Young Children for Lead Poisoning: Guidance for State and Local Public Health Officials*. Atlanta, GA: 1997. [Report]

[3] United States Environmental Protection Agency. *Air Quality Criteria for Lead.* Vol. III; 1986. [Report]

[4] Rabinowitz MB, Wetherill GW, Kopple JD. Kinetic analysis of lead metabolism in healthy humans. *J Clin Invest.* 1976;58:260-270.

[5] Bellinger DC, Stiles KM, Needleman HL. Low-level lead exposure, intelligence, and academic achievement: a long-term follow-up study. *Pediatrics* 90. 1992;855-861.

[6] Stauber JL, Florence TM, Gulson BL, Dale LS. Percutaneous absorption of inorganic lead compounds. *The Science of Total Environ.* 1994;145:55-70.

[7] Pirkle JL, Brody DJ, Gunter EW, et al. The decline in blood lead levels in the United States: The National Health and Nutrition Examination Surveys (NHANES). *JAMA*. 1994;272:284-291.

[8] Ziegler EE, Edwards BB, Jensen RL, Mahaffey KR, Fomon SJ. Absorption and retention of lead by infants. *Pediatr Res.* 1978;12:29-34.

[9] Needleman HL. *Human Lead Exposure*. Boca Raton, FL: CRC Press. 1991:1-287.

[10] National Research Council. *Measuring Lead Exposure in Infants, Children, and Other Sensitive Populations.* Washington, DC: National Academy Press. 1993:31-98.

[11] Chisolm JJ, Jr. Aminoaciduria as the manifestation of retinal tubular injury in lead intoxication and a comparison with patterns of aminoaciduria seen in other diseases. *J Pediatr*. 1962;60:1-17.

[12] Henderson DA. A follow-up of cases of plumbism in children. *Aust Ann Med.* 1954;3:219-224.

[13] Wedeen RP. Lead, the kidney and hypertension. In: Needleman HL, ed. *Human Lead Exposure.* Boca Raton, FL: CRC Press. 1991:169-190.

[14] Anderson AC, Pueschel SM, Linakis JG. Pathophysiology of lead. In: Pueschel SM, Linakis JG, Anderson AC, eds. *Lead Poisoning in Childhood.* Baltimore, MD: PH Brookes Publishing Company. 1996:75-96.

[15] Lane RE. The care of the lead worker. *Br J Ind Med.* 1949;6:125-142.

[16] Paul C. Study of the effect of slow lead intoxication on the product of conception (in French). *Arch General Med*. 1860;15:513-533.

[17] Goldstein GW. Developmental neurobiology of lead toxicity. In: Needleman HL, ed. *Human Lead Exposure.* Boca Raton, FL: CRC Press. 1991:125-136.

[18] Emmerson BT. Chronic lead nephropathy: the diagnostic use of calcium EDTA and the association with gout. *Aust Ann Med.* 1963;12:310-324.

[19] Emmerson BT. Chronic lead nephropathy (editorial). *Kidney Int.* 1973;4:1-5.

[20] Todd AC, Landrigan PJ, Bloch P. Workshop on the X-ray fluorescence of lead in bone: conclusions, recommendations, and summary. *Neurotoxicology*. 1993;14:145-154.

[21] Todd AC, Chettle DR. In vivo x-ray fluorescence of lead in bone: review and current issues. *Environ Hlth Perspect.* 1994;102:172-177.

[22] Clarkson TW, Friberg L, et al., eds. *Biological Monitoring of Toxic Metals*. New York, NY: Plenum Press. 1988:627-640.

[23] Taylor A. Usefulness of measurements of trace elements in hair. *Ann Clin Biochem*. 1986;23:364-378.

[24] Needleman HL, ed. *Human Lead Exposure.* Boca Raton, FL: CRC Press. 1992:54-58.

[25] Manson MB, Zlotkin S. Hair analysis—a critical review. *Can Med Assoc J*. 1985;133:186-188.

[26] Hambridge KM. Hair analysis: worthless for vitamins, limited for minerals. *Am J Clin Nutr.* 1982; 36:943-949.

[27] Gunter EW, Turner WE, Huff DL. Investigation of protoporphyrin IX standard material used in acid-extraction methods, and a proposed correction for the millimolar absorptivity of protoporphyrin IX. *Clin Chem.* 1989;35(8):1601-1609.

[28] Piomelli S, Seaman D, Zullow D, Curren A, Davidow B. Threshold for lead damage to heme synthetics in urban children. *Proc Natl Acad Sci.* 1982;79:3335-3339.

[29] Centers for Disease Control and Prevention. Update: Blood lead levels - United States, 1991-1994. *MMWR*. 1997;46:141-146

[30] Iyengar V, Woittiez J. Trace elements in human clinical specimens: evaluation of literature data to identify reference values. *Clin Chem.* 1988;34:74-481.

[31] Sesana G, Baj A. Determination of lead and cadmium in urine using Zeeman GFAAS. In: Minoia C, Caroli S, eds. *Applications of Zeeman Graphite Furnace AAS in the Chemical Laboratory and in Toxicology*. Pergamon Press. 1992;459-474.

[32] Minoia C, Sabbioni E, Apostoli P, et al. Trace element reference values in tissues from inhabitants of the European community I: a study of 46 elements in urine, blood, and serum of Italian subjects. *Science of the Total Environment.* 1990:95;89-105.

[33] Roels H, Lauwerys R, Konings J, et al. Renal function and hyperfiltration capacity in lead smelter workers with high bone lead. *Occup & Environ Med.* 1994;51:505-512.

[34] Paschal DC, Ting BG, Morrow JC, Pirkle JL, Jackson RJ, Sampson EJ. Metals in urine of United States residents: reference range concentrations. *Envir Res.* January 1998;76:53-59.

[35] Bamback K, Burkey RE. Microdetermination of lead by dithizone (Anal. ed.) *Ind Eng Chem*. 1942;14:904-907.

[36] Blaxter KL, Allcroft R. Lead as a nutritional hazard to farm animals. *J Comp Pathol.* 1950;60:133-139.

[37] Vinter P. Colorimetric determination of blood-lead. *J Med Lab Technol.* 1964;21:281-284.

[38] National Academy of Sciences. *Lead: Airborne Lead in Perspective*. Committee on biologic effects of atmospheric pollutants. Washington, D.C.: Division of Medical Sciences, National Research Council, NAS. 1972:226-248.

[39] Browett EV, Moss R. Manual and semi-automatic methods for the determination of the lead content in urine. *Analyst.* 1965;90:715-726.

[40] Delves HT, Vinter P. Semi-automatic determination of lead in whole blood. *J Clin Path.* 1966;19: 504-509.

[41] Cernik AA. A dry coating method for the determination of lead in blood using cathode ray polarography. *B J Ind Med.* 1967;24:289-293.

[42] Berman E. The determination of lead in blood and urine by atomic absorption spectrophotometry. *Atomic Absorpt News.* 1964;3:111-114.

[43] Hessel DW. A simple and rapid quantitative determination of lead in blood. *Atomic Absorpt News.* 1968;7:55-56.

[44] Kahn HL, Peterson GE, Shallis JE. Atomic absorption microsampling with the "sampling boat" technique. *Atomic Absorpt News.* 1968;7:35-39.

[45] White RA. A microliter sampling system for atomic absorption spectrophotometry. Paper presented at the 2nd International Conference on Atomic Spectroscopy. Sheffield, England; 1969.

[46] Delves HT. A microsampling method for the rapid determination of lead in blood by atomic absorption spectrophotometry. *Analyst.* 1970;95:431-438.

[47] *Annual reports on analytical atomic spectroscopy, Vol I- VIII.* SAC, England; 1971-1978.

[48] Mitchell DG, Aldous KM, Ryan FJ. Automated determination of BPb levels in microsamples using a Delves cup, photon-counting, on-line computer, atomic absorption system. In: Barth D, Berlin A, Engel R, Recht P, Smeets J, eds. *Environmental Health Aspects of Lead: Proceedings of an International Symposium*, October 2-6, 1972. Amsterdam, Luxembourg: Commission of the European Communities. 1973:10813-11089.

[49] Delves HT. Direct read-out of blood-lead concentrations using a simple integration microsampling atomic absorption method. Paper presented at the 4th SAC Conference. Birmingham, England: July 1977.

[50] Koirtyohann SR, Pickett EE. Background correction in long-path AAS. *Anal Chem.* 1965;37:601-603.

[51] Matson WR, Griffin RM, Schreiber GB. Rapid subnanogram simultaneous analysis of Zn, Cd, Pb, Cu, Bi. In: D. Hemphill, ed. *Trace Substances in Environmental Health*. 4th ed. Columbia, MO: University of Missouri. 1971:396-406.

[52] Duic L, Szechter S, Srinivasan SJ. A micro-determination of lead in blood: a derivative pulse stripping voltammetric method. *J Electroanal Chem Interfacial Electrochem*. 1973;41:89-93.

[53] Searle B, Chan W, Davidow B. Determination of lead in blood and urine by anodic stripping voltammetry. *Clin Chem.* 1973;19:76-80.

[54] Copeland TR, Skogerboe RK. Anodic stripping voltammetry. *Anal Chem.* 1974;46:257A.

[55] Morrell G, Giridhar G. Rapid micromethod for blood lead analysis by anodic stripping. *Clin Chem.* 1976;22:221-223.

[56] Pinchin MJ, Newham J. The determination of lead, copper and cadmium by anodic stripping voltammetry at a mercury thin film electrode. *Anal Chim Acta.* 1977;90:91-102.

[57] Osterloh JD, Sharp DS, Hata B. Quality control data for low blood lead concentrations by three methods used in clinical studies. *J Anal Toxicol.* 1990;14:22;8-11.

[58] Bannon DI, Murashchik C, Zapf CR, Farfel MR, Chisolm JJ Jr. Graphite furnace atomic absorption spectroscopic measurement of blood lead in matrix-matched standards. *Clin Chem*. 1994;40:1730-1734.

[59] Amos MD, Bennett PA, Brodie KG, Lung PWY, Matousek JP. Carbon rod atomizer in atomic absorption and fluorescence spectrometry and its clinical applications. *Anal Chem.* 1971;43: 211.

[60] Kubasik NP, Volosin MT, Murray MH. Carbon rod atomizer applied to measurement of lead in whole blood by atomic absorption spectrophotometry. *Clin Chem.* 1972;18:410.

[61] Fernandez F. Micromethod for lead determination in whole blood by atomic absorption, with use of the graphite furnace. *Clin Chem.* 1975;21:558-561.

[62] Rosen JF, Trinidad EE. The microdetermination of blood lead in children by flameless atomic absorption: the carbon rod atomizer. *J Lab Clin Med*. 1972;80(4):567-576.

[63] L'vov BV. *Atomic Absorption Spectrochemical Analysis.* London: Hilger; 1970.

[64] Lundgren G, Lundmark L, Johansson G. Temperature controlled heating of the graphite tube atomizer in flameless AAS. *Anal Chem.* 1974;46:1028-1031.

[65] Ediger RD. Atomic absorption analysis with the graphite furnace using matrix modification. *Atomic Absorpt News*. 1975;14:127-130.

[66] L'vov BV. Electrothermal atomization— the way toward absolute methods of AAS. *Spectrochim Acta, Part B.* 1978;33:153-193.

[67] Slavin W, Manning DC. Reduction of matrix interferences for Pb determination using the L'vov platform and the graphite furnace. *Anal Chem.* 1979;51:261-265.

[68] Slavin W, Manning DC, Carnrick GR. The stabilized temperature platform furnace. *At Spectrosc*. 1981;2:137-145.

[69] Fernandez F, Hilligoss D. Improved graphite-furnace method for determination of lead in blood using matrix modification and the L'vov platform. *At Spectrosc.* 1982;3:130-131.

[70] Shuttler IL, Delves HT. Determination of lead in blood by atomic absorption spectrometry with electrothermal atomisation. *Analyst*. 1986;111:651-656.

[71] Miller DT, Paschal DC, Gunter EW, Stroud PE, D'Angelo J. Determination of lead in blood using electrothermal atomization atomic-absorption spectrometry with a L'vov platform and matrix modifier. *Analyst*. 1987;112:1701-1704.

[72] Subramanian KS. Determination of lead in blood by graphite-furnace atomic-absorption spectrometry—a critique. *Sci. Total Environ.* 1989;89:237.

[73] Shuttler I, Delves HT. Elemental analysis of body fluids and tissues by ETA-AAS. In: Haswell SJ, ed. *Atomic Absorpt Spectrom*. Amsterdam: Elsevier. 1991:381-438.

[74] Parsons PJ, Slavin W. A rapid Zeeman graphite furnace atomic absorption spectrometric method for the determination of lead in blood. *Spectrochim Acta, Part B*. 1993;48:925-939.

[75] Nixon DE, Moyer TP. Routine clinical determination of lead, arsenic, cadmium, and thallium in urine and whole blood by inductively coupled plasma mass spectrometry. *Spectrochim Acta, Part B*. 1996;51:13-25.

[76] Delves HT, Campbell MJ. Measurements of total lead concentrations and of lead isotope ratios in whole blood by use of inductively coupled plasma source mass spectrometry. *J Anal At Spectrom.* 1988;3:343-348.

[77] Campbell MJ, Delves HT. Accurate and precise determination of lead isotope ratios in clinical and environmental samples using inductively coupled plasma source mass spectrometry. *J Anal At Spectrom.* 1989;4:235-236.

[78] Gercken B, Barnes RM. Determination of lead and other trace element species in blood by size exclusion chromatography and inductively coupled plasma/mass spectrometry. *Anal Chem*. 1991; 63:283-287.

[79] Paschal DC, Caldwell KL, Ting BG. Determination of lead in whole blood using inductively coupled argon plasma mass spectrometry with isotope dilution. *J Anal At Spectrom.* 1995;10:367-370.

[80] Bowins RJ, McNutt RH. Electrothermal isotope dilution inductively coupled plasma mass spectrometry method for the determination of sub-ng mL^{1} levels of lead in human plasma. *J Anal At Spectrom.* 1994;9:1233-1236.

[81] Nuttall KL, Gordon WH, Ash KO. Inductively coupled plasma mass spectrometry for trace element analysis in the clinical laboratory. *Ann Clin Lab Sci.* 1995;25:264-271.

[82] Velapoldi RA, Paule RC, Schaffer R, Mandel J, Machlan LA, Gramlich JW. *Standard Reference Materials: A reference method for the determination of potassium in serum*. NBS Special Publication 260-63. Washington, DC: U.S. Dept. of Commerce. 1979:104.

[83] Velapoldi RA, Paule RC, Schaffer R, Mandel J, Murphy TJ, Gramlich JW. *Standard Reference Materials: A reference method for the determination of chloride in serum*. NBS Special Publication 260-67. Washington, DC: U.S. Dept. of Commerce. 1979:97.

[84] Velapoldi RA, Paule RC, Schaffer R, Mandel J, Machlan LA, Garner EL, Rains TC. *Standard Reference Materials: A reference method for the determination of lithium in serum*. NBS Special Publication 260-69. Washington, DC: U.S. Dept. of Commerce. 1980:114.

[85] Bowers GN, Fassett JD, White E. Isotope dilution mass spectrometry and the national reference system. *Anal Chem*. 1993;65(12):475R-479R.

[86] Moody JR, Epstein MS. Definitive measurement methods. *Spectrochim Acta.* 1991;46B(12):1571-1575.

[87] Fassett JD, Paulson PJ. Isotope dilution mass spectrometry for accurate elemental analysis. *Anal Chem*. 1989;61:643A-649A.

[88] Patterson CC, Settle DM. In: LaFleur PD, ed. *Accuracy in Trace Analysis: Sampling, Sample Handling and Analysis.* Vol 1. NBS Special Publication 422. Gaithersburg, MD. 1976.

[89] Yaffe Y. Identification of lead sources in California children using the stable isotope ratio technique. *Archives of Environmental Health*. 1983;38(4):237-245.

[90] Wang J, Tian BM. Gold ultramicroelectrodes for on-site monitoring of trace lead. *Electroanalysis*. 1993;5:809-814.

[91] Wang J, Sucman E, Tian BM. Stripping potentiometric measurements of copper and blood using gold microelectrodes. *Anal Chim Acta*. 1994;286:189-195

[92] Wang J, Lu J, Tian B, Yarnitsky C. Screen-printed ultramicroelectrode arrays for on-site stripping measurements of trace metals. *J Electroanal Chem.* 1993;361:7-83.

[93] Feldman BJ, D'Alessandro A, Osterloh JD, Matta BH. Electrochemical determination of low blood lead concentrations with a disposable carbon microarray electrode. *Clin Chem.* 1995;41:557-563.

[94] Parsons PJ, Qiao H, Adlous KM, Mills E, Slavin W. A low-cost tungsten filament atomizer for measuring lead in blood by atomic absorption spectrometry. *Spectrochim Acta, Part B*. 1995;50: 1475-1480.

[95] Wensing MW, Smith BW, Winefordner JD. Capacitively coupled microwave plasma atomic emission spectrometer for the determination of lead in whole blood. *Anal Chem*. 1994;66:531-535.

[96] Wensing MW, Liu DY, Smith BW, Winefordner JD. Determination of lead in whole blood using a capacitively coupled microwave plasma atomic emission spectrometer. *Analytica Chimica Acta*. 1994:299.

[97] Centers for Disease Control and Prevention. Preventing lead poisoning in young children: a statement by the Centers for Disease Control. CDC Report No. 99-2230. Atlanta, GA: CDC; 1985.

[98] De Silva PE, Donnan MB. Blood lead levels in Victorian children. *Med J Aust.* 1980;1:93.

[99] Mitchell DG, Aldous KM, Ryan FJ. Mass screening for lead poisoning: capillary blood sampling and automated Delves-cup atomic-absorption analysis. *NY State J Med*. 1974;74:1599-1603.

[100] Sinclair DF, Dohnt BR. Sampling and analysis techniques used in a blood lead survey of 1241 children in Port Pirie, South Australia. *Clin Chem*. 1984;10:1616-1619.

[101] New England Consortium of Childhood Lead Poisoning Programs. New England Public Health Laboratory Lead Testing Services [report]. Providence, RI: NECCLPP; 1985.

[102] Kehoe RA. The metabolism of lead in man in health and disease. *Arch Environ Health*. 1961;2:418-422.

[103] Lyngbye T, Jorgensen PJ, Grandjean P, Hansen ON. Validity and interpretation of blood lead levels: a study of Danish school children. *Scand J Clin Lab Invest*. 1990;50:441-449.

[104] Parsons PJ, Reilly AA, Esernio-Jenssen D. Screening children exposed to lead: an assessment of the capillary blood lead fingerstick test. *Clin Chem.* 1997;43:302-311.

[105] Schonfeld DJ, Cullen MB, Rainey PM, Berg AT, Brown DR, Hogan JC, Turk DS. Screening for lead poisoning in an urban pediatric clinic using samples obtained by fingerstick. *Pediatrics*. 1994;2:74-179.

[106] Schlenker TL, Fritz CJ, Mark D, Layde M, Linke G, Murphy A, Matte T. Screening for pediatric lead poisoning: comparability of simultaneously drawn capillary and venous blood samples. *JAMA*. 1994;271:1346-1348.

[107] Rainey PM, Schonfeld DJ. Comparability of capillary and venous blood samples for lead screening. *JAMA*. 1994;272:1482.

[108] Cernik AA, Sayers MHP. Determination of lead in capillary blood using a paper punched disc atomic absorption technique. *Br J Industr Med.* 1971;28:392-398

[109] Stanton NV, Maney JM, Jones R. Evaluation of filter paper blood lead methods: results of a pilot proficiency testing program. *Clin Chem.* 1999;45(12)**:**2229-2235.

[110] Verebey K, Rosen JF, Schonfeld DJ, Carriero D, Eng YM, Deutsch J, Reimer S, Hogan J. Blood lead and analytical considerations in blood lead screening in children. *Clin Chem.* 1995;41:469-470.

[111] O'Broin SD, Kelleher BP, Gunter E. Evaluation of factors influencing precision in the analysis of samples taken from blood spots on filter paper. *Clin Lab Haem.* 1995;17:185-188.

[112] Carter GF. The paper punched disk technique for lead in blood samples with abnormal haemoglobin values. *Brit J Ind Med.* 1978;35:235-240.

[113] Delves HT. Blood collection for screening children for exposure to lead. *Clin Chem*. 1996;42:983-984.

[114] Moyer TP, Nixon DN, Ash KO. Filter paper lead testing. *Clin Chem.* 1999;45(12)**:**2055-2056.

[115] Verebey K. Filter paper-collected blood lead testing in children. *Clin Chem.* 2000;46:1024-1026.

[116] Moyer TP, Nixon DE, Ash KO. *Clin Chem* 2000;46:1026-1028. Reply to: Filter paper-collected blood lead testing in children. *Clin Chem.* 2000;46:1026-1028.

[117] Stanton N, Maney J, Jones R. More on filter paper lead testing. *Clin Chem.* 2000;46:1028-1029.

[118] Verebey K. Blood lead screening (continued). *Clin Chem*. 1997;43:1247-1248.

[119] Mehkeri KA, Romanowski M, Smallbone B. Use of a filter disk micro-sampling atomic absorption method for blood lead level screening. *Am Ind Hyg Assoc J.* 1976;37:541-545.

[120] Cernik AA. Determination of blood lead using a 4.0 mm paper punched disk carbon sampling cup technique. *Brit J Ind Med.* 1974;31:239-244.

[121] Verebey K. Blood collection for screening children for exposure to lead (reply). *Clin Chem.* 1996;42:984-985.

[122] Wang S, Demshar HP. Determination of blood lead in dried blood-spot specimens by Zeeman-effect background corrected atomic absorption spectrometry. *Analyst.* 1992;117:959-961.

[123] Holtrop TG, Yee HY, Simpson PM, Kauffman RE. A community outreach lead screening program using capillary blood collected on filter paper [published erratum appears in *Arch Pediatr Adolesc Med*. 1998 Oct;152(10):991]. *Arch Pediatr Adolesc Med.* 1998;152(5)**:**455-458.

[124] Srivuthana K, Yee HY, Bhambhani K, Elton RM, Simpson PM, Kauffman RE. A new filter paper method to measure capillary blood lead level in children. *Arch Pediatr Adolesc Med.* 1996;150(5)**:**498-502.

[125] Schonfeld DJ, Cullen MR, Rainey PM, Berg AT, Brown DR, Hogan JC, Turk DS, Rude CS, Cicchetti DV. Screening for lead poisoning in an urban pediatric clinic using samples obtained by fingerstick. *Pediatrics.* 1994;94:174-179.

[126] Yee HY, Holtrop TG. An improved capillary blood-filter paper-graphite furnace atomic absorption spectrophotometric method for lead screening. *J Anal Tox*. 1997;21:142-148.

[127] Verebey K, Eng YM, Davidow B, Ramon A. Rapid, sensitive micro blood lead analysis: a mass screening technique for lead poisoning. *J Anal Tox*. 1991;15:237-240.

[128] Kaul B, Mukerjee H. Elevated Blood Lead and Erythrocyte Protoporhyrin levels of children near a battery recycling plant in Haina, Dominican Republic, *Int J Occup.* Environ Health. 1999;5:307-312.

[129] Kaul B. Lead Exposure and iron deficiency among Jammu and New Delhi children, *J Pediatr*. 1999; 66:27-35.

[130] NYSDH. *Blood Lead and Erythrocyte Protoporphyrin: A Recommended Procedure for Collecting Fingerstick Blood Specimens*. Albany, NY: New York State Department of Health, Wadsworth Center for Laboratories and Research. 1989;1-14.

[131] WSLH. *Complete Sampling Instructions for Capillary Lead.* Madison, WI: Wisconsin State Laboratory of Hygiene, University of Wisconsin Center for Health Sciences; 1985.

[132] MHD. *Generalized Procedure Fingerstick Blood (hematocrit and/or lead test)*. Milwaukee, WI: Milwaukee Health Department; 1988.

[133] CDHS. *Childhood Blood Lead Screening: Fingerstick Blood Sampling Method.* Berkeley, CA: California Department of Health Services; 1990.

[134] MDPH. *Procedure for Obtaining Fingerstick Blood Samples.* Jamaica Plain, MA: Massachusetts Department of Public Health, Childhood Lead Poisoning Program; 1990.

[135] Wang ST, Peter F. Stability of human blood lead in storage. *J Anal Toxicol.* 1985;9:85-88.

[136] WHO. Technical Report Series No. 647. Recommended health-based limits in occupational exposure to heavy metals report of a WHO study group. Geneva, Switzerland: World Health Organization; 1980.

[137] Pruszkowska E, Carnrick GR, Slavin W. Blood lead determination with the platform-furnace technique. *At Spectrosc.* 1983;4:59-61.

[138] Qiao H, Parsons PJ, Slavin W. Transferability of a simple method for blood lead determination using furnace atomic absorption spectrometry and continuum background correction. *Clin Chem*. 1995;41:1451-1454.

[139] Flajnik CE, Shrader D. Determining lead in blood: evaluating deuterium and Zeeman background correction. *Am Clin Lab.* 1994;13(9):45-46.

[140] Helfrich JP. A workstation-based quick furnace method for the determination of lead in whole blood. *Am Clin Lab.* 1994;13(4):14-15.

[141] Subramanian KS, Meranger JC, MacKeen JE. Graphite furnace atomic absorption spectrometry with matrix modification for determination of cadmium and lead in human urine. *Anal Chem.* 1983;55:1064-1067.

[142] Paschal DC, Kimberly MM. Determination of urinary lead by electrothermal atomic absorption with the stabilized temperature platform furnace and matrix modification. *At Spectrosc*. 1985;6:134-136.

[143] Lian L. Direct determination of lead in urine by Zeeman atomic absorption spectrometry with the stabilized temperature platform furnace. *Spectrochim Acta, Part B.* 1986;41:1131-1135.

[144] Sesana G, Baj A. Determination of lead and cadmium in urine using Zeeman GFAAS. In Minoia C, Caroli S, eds. *Applications of Zeeman Graphite Furnace AAS in the Chemical Laboratory and in Toxicology*. Pergamon Press. 1992;459-474.

[145] Parsons PJ, Slavin W. Electrothermal atomization atomic absorption spectrometry for the determination of lead in urine: results of an interlaboratory study. *Spectrochim Acta, Part B*. 1999; 54:853-864.

[146] Miller JC, Miller JN. *Statistics for Analytical Chemistry*. 2nd. ed. Chichester, UK: Ellis Horwood Ltd; 1988:44.

[147] Jacobson BE, Lockitch G, Quigley G. Improved sample preparation for accurate determination of low concentrations of lead in whole blood by graphite furnace analysis. *Clin Chem*. 1991;37:515-519.

[148] Quantitative trace analysis of biological materials. In: McKenzie HA, Smythe LE, eds. *Polarography and Stripping Voltammetry*. Amsterdam, New York, London: Elsevier; 1988: chap 12.

[149] Peters DG, Hayes JM, Hieftje GM. Chemical separations and measurements. In: *Polarography and Amperometric Titrations.* Philadelphia, London, Toronto: Saunders; 1974: chap 13.

[150] Bannon DI, Murashchik C, Zapf CR, Farfel MR, Chisolm JJ, Jr. Graphite furnace atomic absorption spectroscopic measurement of blood lead in matrix-matched standards. *Clin Chem*. 1994;40:1730-1734.

[151] ESA Laboratories, Inc. *Model 3010B lead analyzer operating manual.* Chelmsford, MA: ESA, Inc; 1994.

[152] Roda SM, Greenland RD, Bornschein RL, Hammond PB. Anodic stripping voltammetry procedure modified for improved accuracy of blood lead analysis. *Clin Chem.* 1988;34:563-567.

[153] ISO 8402. *Management and Quality Assurance Vocabulary*. Geneva, Switzerland: International Organization for Standardization; 1986.

[154] Uriano GA, Gravatt CC. The role of reference materials and reference methods in chemical analysis. *CRC Critical Reviews in Analytical Chemistry.* October 6, 1977:361-411.

[155] Moody JR, Epstein MS. Definitive measurement methods. *Spectrochim Acta*. 1991;46B(12):1571-1575.

Appendix A. Procedure for Checking Materials and Specimen Collection Supplies for Lead Contamination

A1 Procedure for Blood-Collection Tubes

A1.1 Scope and Application

The laboratory is responsible for assuring lead-free collection materials. The laboratory may either supply suitable collection materials that it has proven to be lead-free or recommend suitable sources to providers.

The laboratory should establish a dialogue with the submitters to assure that contamination is kept to a minimum. It is suggested that the laboratory offer in-services periodically to submitters. If this is not feasible, contact the state or local health laboratory for assistance through the regional NLTN (National Laboratory Training Network; a cooperative agreement between CDC and The Association of Public Health Laboratories.)

The purpose of this procedure is to test blood collection tubes and other materials for gross Pb contamination. Collection devices such as evacuated tubes and microcollection tubes are tested by measuring the amount of lead leached into a blood pool that has a low but well-characterized lead content. This approach is considered closest to the situation in which routine blood specimens are collected and transported to the laboratory, and stored pending analysis. Alternatively, tubes may be filled with 1% (v/v) nitric or 4% acetic acid solution, which can leach lead into solution.

A1.2 Summary of Procedure Using Blood

Sample containers are filled with a reference blood sample of low Pb level (such as a normal, nondosed animal blood) to the minimum fill volume. Blood samples are stored refrigerated for ten days, i.e., for the upper limit expected between specimen collection and analysis, if the specimen is stored appropriately. The blood samples are analyzed for Pb.

A1.3 Interferences

This method has no interferences; however, extraction may be incomplete.

A1.4 Preparation

(1) Randomly select ten blood-collection tubes from a given batch or lot number. Fill each of them with blood of a low-lead concentration to the minimum volume required for the test, i.e., to generate the highest Pb concentration from any contamination.

(2) Refrigerate for ten days at 2 to 8 °C.

A1.5 Analysis of Blood for Lead

Aliquots of blood from each tube and the original blood pool are analyzed for lead.

A1.6 Calculations

To calculate the amount of contaminant lead leached from the sample containers, subtract the average BPb value of the original blood pool from the mean of the values from the blood-filled tubes to find the mean difference.

A1.7 Summary of Procedure Using Acidic Solution

Sample containers are filled to the minimum fill volume with a 1% nitric or 4% acetic acid solution. Samples are then stored at room temperature for at least 24 hours, and analyzed for lead content.

A1.8 Preparation

(1) Randomly select at least ten collection containers from each lot to be tested. Fill each with the acidic solution to the minimum volume required for testing.

(2) Store containers for at least 24 hours at room temperature. Containers should be stored on their sides to ensure contact between the solution and the caps of the containers.

(3) Analyze the solution from each container for lead content. Determine the mean lead concentration.

A1.9 Interpretation of Results and Definition of Significant Lead Contamination

Containers should be considered suitable for use in lead testing when the mean lead concentration or difference in BPb is ≤0.5 µg/dL. Concentrations greater than this threshold indicate unacceptable lead contamination, and the containers should not be used for BPb testing.

Any single measurement exceeding 0.7 µg/dL may also indicate unacceptable contamination. A second set of containers should then be tested. If the second set of containers also produces one or more measurements exceeding 0.7 µg/dL, the lot should be considered unacceptable for use in BPb testing.

A.2 Procedure for Testing Other Collection Materials

A2.1 Scope and Application

In addition to collection containers, it may be useful to test other collection components, e.g., needles, lancets, and alcohol swabs. Lancets and needles are tested by measuring the amount of lead leached into a solution of 4% (v/v) acetic acid or 1% (v/v) nitric acid; this is based upon a recommended FDA procedure for checking dishes and hollow ceramicware for lead contamination.[1] Alcohol swabs can be tested by direct measurement of the alcohol solution.

A2.2 Summary of Method

The solution from alcohol swabs is expressed directly into autosampler cups or other lead-free containers. Other materials are soaked in dilute acetic or nitric acid. The amount of lead in the leachate or alcohol solution is determined by a standard analytical method.

A2.3 Interferences

This method has no interferences; however, extraction may be incomplete.

A2.4 Apparatus and Materials

- Covered vessel for testing materials.

- 4% (v/v) acetic acid: Fill a 1-L graduated cylinder with 500 mL of deionized water suitable for trace element work, i.e., resistivity >10 MΩ · cm. Under a fume hood, slowly add 40 mL glacial acetic acid (ultrapure grade) and dilute to 1 L. The diluted reagent should not be used if it is more than one week old.

- 1% HNO_3 (v/v)

A2.5 Sample Preparation

Choose at least ten units from each lot of materials to be tested. For all materials except alcohol swabs, immerse the objects in the minimum volume of acid necessary to cover them. Record the volume of acid used. Cover the vessel with plastic wrap to avoid evaporation and airborne contamination, and maintain for 24 hours at room temperature.

To test alcohol swabs, snip off a corner of each packet, and by squeezing the packet, express the alcohol solution directly into an autosampler cup or other suitable lead-free container. The solution should then be tested as soon as possible to minimize the possible effect of evaporation.

A2.6 Analysis for Lead

Analyze the alcohol solution directly for lead content.

Analyze the leachate and the "blank" acid solution for Pb. The "blank" acid should not contain detectable lead.

A2.7 Calculations

Determine the mean lead concentration (µg/dL) in the alcohol solution.

Calculate the amount (µg) of lead (if any) leached from the other materials, by multiplying the w/v concentration by the measured volume of leaching solution used.

A2.8 Interpretations

Alcohol swabs are considered acceptable if the mean lead concentration of the alcohol solution is <0.5 µg/dL, and no single measurement exceeds 0.7 µg/dL (see Section A1.9).

For other tested materials, ideally there should be no detectable lead in the leachate. If a measurable amount of lead is detected, the test should be repeated. If measurable lead is still detected after repeat testing, that batch of swabs should not be used for lead-screening purposes.

Reference to Appendix A

1. AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists.* 13th ed. Washington, DC: Association of Official Analytical Chemists. 1980;25:031-034.

Appendix B. Laboratory Certification and Proficiency Testing in the United States

Certification of all U.S. laboratories performing BPb testing is currently required under the Clinical Laboratory Improvement Amendments (CLIA '88). Laboratories testing for possible occupational exposure to lead must also maintain approval from the Occupational Safety and Health Administration (OSHA). In addition, an increasing number of states also regulate BPb laboratories, and one or more of these state-level certifications may also be needed. Requirements for these various certification processes differ, but federal and most state regulations include a requirement for successful participation in an approved proficiency testing (PT) program.

B1 CLIA '88

The CLIA '88 regulations, published in 1992 (42 CFR, Part 405 et al.), are administered by the Health Care Financing Administration (HCFA), and define minimum standards for virtually all aspects of laboratory operation, including personnel qualifications, physical plant, specimen handling, reporting and record keeping, etc. Certification under CLIA is required for all U.S. laboratories performing BPb testing, with the exception of forensic and research laboratories. Successful performance in an approved PT program is required. Currently, four HCFA-approved PT programs are available. A list of these programs can be found in Section B4.

The approved PT programs must provide three yearly testing events of five samples each (the federal MCHB/WSLH program also provides monthly nonregulatory testing events). Sample acceptability limits are defined as the target value ±4 µg/dL or 10%, whichever is larger. Satisfactory CLIA performance is defined as a score of ≥80% (4/5) acceptable results in a testing event. If two of three consecutive testing events result in unsatisfactory scores, performance is considered unsuccessful, and revocation of the certification may result.

B2 OSHA Approval

U.S. laboratories testing blood obtained for occupational screening must obtain OSHA approval in addition to CLIA certification. Administered by OSHA since 1979, approval is based exclusively on PT performance. However, the criteria for approval are significantly different from those specified under CLIA. Using data from an OSHA-approved PT program, OSHA defines sample acceptability limits as the sample target value ±6 µg/dL or 15%. Because these criteria are 50% wider than those defined by CLIA and employed by the approved PT programs, a reinterpretation of the PT data is required. Evaluation using these limits requires that labs must obtain acceptable results on >89% of the PT challenges for the most recent 12-month period. There is no proration for shorter time periods of data accumulation. OSHA updates their data and publishes a list of approved laboratories three times yearly.

Data from four PT programs can be used for OSHA evaluation purposes. Information on these programs can also be found in Section B4.

B3 State-Level Certifications

Certification at the state level has seen significant growth in the 1990s, and many states now have statutes establishing requirements for BPb laboratories. These requirements can vary substantially, but most state certification programs include a PT component and mandatory reporting requirements. Some states, e.g., New York and Pennsylvania, administer their own PT programs, while most accept data from one or more of the HCFA-approved PT providers. State health departments from all states comprising a laboratory's specimen base should be contacted to ensure compliance with state certification requirements.

Though now commonly associated with laboratory regulation, PT was originally utilized as a tool for the improvement of data quality. The frequency of testing, method of target value assignment, the type and amount of performance statistics provided, and other aspects vary among programs. Voluntary participation is allowed in several PT programs, and enrollment in more than one PT program is recommended for maximum QC benefit.

B4 Proficiency Testing Programs for Blood Lead—United States and Canada

Country **CANADA**

Organization

Le Centre de Toxicologie du Québec
Programme de Comparisons Interlaboratoires
Laboratorie de Toxicologie - CHUL
2705 Boul. Laurier
Sainte-Foy, Qc
Canada G1V-4G2

Contact person: Dr. Jean-Phillipe Weber
Tel 418-654-2100. Fax 418-654-2148.
e-mail: ctqlab@riq.qc.ca
Website: www.ctq.qc.ca/pciendes.html

Status of Program

International, voluntary.

Aim: To promote improvement of laboratory performance for blood lead (and other trace elements in biological samples).

Accreditation: Approved by the U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) for occupational screening laboratories in the U.S.

Participants in 1997: 92

Program Description[1]

PT materials: Prepared in-house by pooling human blood from exposed workers; blood is preserved with EDTA; target values are the all-method mean of a group of 6 to 10 referee laboratories.

Organization of PT events: Samples are sent six times a year, three samples per batch.

Elaboration of results: Reports include all participant results by code #, frequency distribution graphs, and summary statistics.

Criteria for evaluation of laboratory performance: Cumulative scoring based on proximity to the target value.

Measures taken against poor performance: None.

Advice and training: Available by personal contacts when requested.

Financial support: Supported by laboratory fees.

Analytes and matrices covered

Whole blood: Pb, Cd, Hg
Serum: Al, Se, Cu, Zn
Urine: As, Cd, Hg, Cr, Cu, Pb, Se

Country

U.S.A.

Organization

College of American Pathologists (CAP)
325 Waukegan Road
Northfield, IL 60093-2750 U.S.A.

Contact person: Survey Coordinator
Tel 1-800-323-4040
Website: www.cap.org

Status of Program

National, voluntary, open to any laboratory.
Administered in conjunction with the American Association for Clinical Chemistry (AACC).

Aim: To provide accreditation agencies with laboratory data for U.S. certification purposes.

Accreditation: Program is deemed approved by the U.S. Health Care and Financing Administration (HCFA) under CLIA '88; is approved by the U.S. Department of Labor, Occupational Safety and Health Administration (OSHA).

Participants in 1997: 338

Program Description

PT materials: Prepared from EDTA-preserved bovine blood; sample enrichment is obtained by addition of lead nitrate; samples stored frozen in glass vials. Target values are the participant method mean.

Organization of PT events: Samples are sent three times a year per CLIA, five samples per batch.

Elaboration of results: Reports limited to a single participant laboratory's results, frequency distribution graphs, and summary statistics tables.

Criteria for evaluation of laboratory performance: Per CLIA, ±4 μg/dL or ±10%, whichever is greater based on 90% consensus of participants.

Measures taken against poor performance: None.

Advice and training: None.

Financial support: Supported by participant fees.

Analytes and matrices covered

Whole blood: Pb
Serum: Al, Cr, Se, Cu

Country **U.S.A.**

Organization

Commonwealth of Pennsylvania
Department of Health
Bureau of Laboratories
P.O. Box 500
Exton, PA 19341-0500 U.S.A.

Contact person: Ms. Joyce Mayo
Tel 610-363-8500. Fax 610-436-3346.

Status of Program

Statewide, compulsory for all laboratories in Pennsylvania, and some out-of-state laboratories; restricted to laboratories testing Pennsylvania residents.

Aim: To monitor and improve laboratory performance for blood lead, and to certify blood lead laboratory proficiency for state and federal certification purposes.

Accreditation: Program is deemed approved by the U.S. Health Care and Financing Administration (HCFA) under CLIA; and by the Pennsylvania Department of Health.

Participants in 1997: 60

Program Description

PT materials: Prepared in-house from sonicated human blood; sonicated blood treated with heparin and EDTA; sample enrichment is obtained by addition of lead nitrate; target values are the all-method mean of a group of at least ten referee laboratories.

Organization of PT events: Samples are sent three times a year per CLIA, five samples per batch.

Elaboration of results: Reports include an individualized summary of the last three PT events, and summary statistics.

Criteria for evaluation of laboratory performance: Per CLIA, ±4 µg/dL or ±10%, whichever is greater based on 90% consensus of referee pool.

Measures taken against poor performance: Unsatisfactory performance triggers removal of approved status to test patient specimens.

Advice and training: Available by personal contacts when requested.

Financial support: Supported by laboratory fees.

Analytes and matrices covered Blood lead and erythrocyte protoporphyrin

Country **U.S.A.**

Organization

New York State Department of Health
Wadsworth Center
P.O. Box 509
Albany, NY 12201-0509

Contact person: Dr. Patrick J. Parsons
Tel 518-474-5475. Fax 518-473-2895
e-mail: lead@wadsworth.org

Status of Program National, international, compulsory for all laboratories in New York State, and some out-of-state/Canadian laboratories analyzing specimens originating from New York. Open to a limited number of foreign reference laboratories and others. Administered by the Wadsworth Center's Lead Poisoning/Trace Elements Laboratory for the Health Department's Clinical Laboratory Evaluation Program.

Aim: To monitor and improve laboratory performance for blood lead, and to certify blood lead laboratory proficiency for state and federal certification purposes in the U.S.

Accreditation: Program is deemed approved by the U.S. Health Care and Financing Administration (HCFA) under CLIA '88; is approved by the U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) for occupational screening laboratories; and by some other state agencies.

Participants in 1997: 115

Program Description[2] **PT materials**: Prepared in-house from lead-dosed goats; whole blood anticoagulated with EDTA, unfrozen, and shipped in glass tubes. Target values are the all-method mean of a group of at least ten referee laboratories.

Organization of PT events: Samples are sent three times a year per CLIA, five samples per batch.

Elaboration of results: Reports include all participant results by code #, summary statistics, and individualized summary of the last three PT events.

Criteria for evaluation of laboratory performance: Per CLIA, ±4 µg/dL or ±10%, whichever is greater based on 90% consensus of referee pool.

Measures taken against poor performance: Unsatisfactory performance triggers removal of approved status to test patient specimens, and automatic enrollment into a remediation program.

Advice and training: Available by personal contacts when requested or when required under a remediation program.

Financial support: Supported by state government through clinical laboratory permit fees.

Analytes and matrices covered Blood lead and erythrocyte protoporphyrin

Country **U.S.A.**

Organization

Wisconsin State Laboratory of Hygiene
Toxicology Section
2601 Agriculture Drive
P.O. Box 7996
Madison, WI 53707 U.S.A.

Contact person: Program Supervisor
Tel 608-224-6252. Fax 608-224-6259.
e-mail: toxpt@slh.wisc.edu
Website: www.slh.wisc.edu/pt/

Status of Program National, international, voluntary. Open to any laboratory, including non-U.S. Administered by the Wisconsin State Laboratory of Hygiene under a grant from the U.S. Department of Health and Human Services.

Aim: To improve laboratory performance for blood lead in the U.S., and to provide accreditation agencies with laboratory data for certification purposes.

Accreditation: Program is deemed approved by the U.S. Health Care and Financing Administration (HCFA) under CLIA '88; is approved by the U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) for occupational screening laboratories; is approved for College of American Pathology (CAP) accreditation purposes; and by some state agencies.

Participants in 1997: 300

Program Description[3] **PT materials:** Prepared in-house from lead-dosed cows; whole blood anticoagulated with EDTA, and stored frozen in plastic vials. Target values are the all-method mean of a group of at least ten referee laboratories.

Organization of PT events: Samples are sent every month, three samples per batch, except for three events when the number is increased to five.

Elaboration of results: Monthly reports include all participant results, frequency distribution graph, and summary statistics.

Criteria for evaluation of laboratory performance: Per CLIA, ±4 μg/dL or ±10%, whichever is greater, based on 90% consensus of referee pool.

Measures taken against poor performance: None.

Advice and training: Given by personal contacts when requested.

Financial support: No cost to participants; supported by grant MCJ-551003 from the Maternal & Child Health Bureau, Health Resources and Services Administration, U.S. Department of Health and Human Services.

Analytes and matrices covered Blood lead and erythrocyte protoporphyrin

B5 Proficiency Testing* Programs for Blood Lead —Australia/European Union†

Country **Australia**

Name of Program QCT Biological Trace Elements Quality Assurance Programs.

Status of Program International, voluntary.

Aim: To provide a mechanism for ongoing optimization of the quantitative analysis of trace analysis in biological and environmental matrices.

Participants in 2000: 100 Program.

Program description **Material**: irradiated porcine blood, various animal sera, human urine, with and with out additions.

Organization: 3 bloods per month, 2 sera per month, 1 urine per month

Results: Participant results relative to consensus mean, calculation of bias and precision performance indicators, histograms, long-term performance charts.

Criteria for Evaluation: Internally developed reference standard deviations based on historic industry performance

Advice and training: participants are encouraged to contact the program to seek advice on training and other support.

Financial support: participant fees.

Analytes and matrix covered:
Blood: Pb, Cd, As, Se, Hg, Mn
Urine: Cu, Cd, As, Pb, Hg, Fe, pH, Cr, Tl, Co, Sb, Pt, Na, K, Ca, Mg, Creatinine, Al, Se, Ni, Mn, Cl
Serum: Cu, Zn, Se, Al, Pt, Au, ca, Mg, Mn, Cr

Organization:
Quality Control Technology P/L
PO Box 369
Charlestown, NSW 2290
Australia
Tel 61 2 4947 8730
Fax 61 2 4647 8730
Email gwaller@hunterlink.net.au

* Also known as external quality assessment schemes (EQAS).

† Reproduced with permission from European external quality assessment schemes in occupational and environmental medicine. *Annali dell' Istituto Superiore di Sanità.* Vol. 32, No. 2: 1996 and *United Kingdom National External Quality Assessment Schemes.* 3rd ed. August 1997.

Country	**Belgium**
Name of Program	Quality Control Belgium (QCB).
Status of Program	National, voluntary. Run by the Epidemiological Unit of the Institute of Hygiene and Epidemiology. **Aim:** The aim is on one hand to help laboratories to improve the quality of their results and on the other hand to provide national authorities with information on the dispersion presently encountered among the Belgian laboratories. **Participants in 1993:** lead analysis no.=37; cadmium analysis no.=28. **Participants in 1994:** lead analysis no.=27; cadmium analysis no.=23; selenium analysis no.=8.
Program description	**Control materials:** Prepared in-house, from a human matrix (blood from blood donors); multielemental; sample enrichment is obtained by addition of inorganic lead, cadmium, and selenium; liquid samples are stored frozen in plastic vials, -18 °C. The target values are obtained from the regression line calculated from the results of a reference laboratory plotted against the added amounts. **Internal quality control samples:** No provision of calibrators/internal quality control samples. **Organization of EQA exercises:** Samples are sent every three months, three samples per batch. The results are returned within one month, by mail. **Elaboration of results:** Reports include a frequency distribution graph together with: number of results (after trim), mean (µg/l), SD (µg/l), CV (%), median (µg/l), range before trim (µg/l), range after trim (µg/l), the laboratory results. **Criteria for evaluation of laboratory performance:** None. **Measures taken against poor performers:** None. **Advice and training:** Given by personal contacts when asked for. **Financial support:** A financial contribution is requested from each laboratory, and the program is partly supported by national financing (towards salary of a person involved in the program).
Organization	Institute of Hygiene and Epidemiology Rue Juliette Wystman, 16 B-1050 Brussels, Belgium Contact Person: Pierre De Plaen Tel 32-2/6425024. Fax 32-2/6425410 e-mail: pierre.de.plaen@ihe.be
Analyses and matrices covered	Blood lead, blood cadmium, and blood selenium.

Country	**Denmark**
Name of Program	Danish external quality assessment scheme (DEQAS).
Status of Program	International, voluntary. Run by the National Institute of Occupational Health, Denmark.
	Aim: To provide external quality control for laboratories in the occupational health sector in Denmark.
	Participants: varying number (19 in 1994). Analytical chemical laboratories.
Program description	**Control materials:** Usually prepped "in-house." Samples enriched by "spiking." Blood samples are freeze-dried. Target values usually determined as consensus mean values or by one or more reference laboratories if only a few laboratories participate. These two methods of establishing the target values are optional in the PC-programme that makes the statistical elaboration.
	Organization of EQA. Exercises: Frequency of exercises: two per year: number of samples: five at different concentration levels: time schedule for returning the results: within two weeks.
	Evaluation of results: By means of a PC-programme. Reports and control sheets are generated automatically, and returned to the laboratories within two weeks.
	Criteria for evaluation of laboratory performance: Z-score plot, Youden plot, ratio plot, MEP plot and RMSE ½ plot.
	Measures taken against poor performers: Advice is given in the report. No other measures.
Organization	National Institute of Occupational Health Att: Kirsten Byrialsen Lersø Parkallé 105 DK-2100 Copenhagen, Denmark Tel +45 39 29 97 11. Fax + 45 39 27 01 07. email: amilpost@uts.uni.c.dk
Analytes and matrices covered	Lead in human blood. Iron, manganese, and titanium in welding fume. Organic solvents on charcoal tubes.
Country	**France**
Name of Program	Contrôle national de qualité de plombémie.
Status of Program	National, compulsory. Run by the "Agence du médicament-Unité Contrôle National de Qualité."
	Aim: To obtain approval for blood lead analysis.

Participants: 66 hospital, university, and private laboratories.

Program description

Control samples: Human blood samples with or without lead supplementation, prepared in house. Target value: mean of results after exclusion of outliers (data outside the interval: mean ± 2 SD).

Organization of EQA exercise: Three exercises beginning in 1995, including three samples each (nine samples per year). Mail distribution: a two-week delay for sending in results by mail or fax.

Elaboration of results and evaluation of laboratory performance: Evaluation of the results in terms of comparison to the target value and recovery of the added amounts. Scores and acceptability limits as defined by Taylor & Briggs.[4,5]

Measures against poor performers: Laboratories not obtaining the average are refused ministerial approval.

Financial support: A fee is charged: 2,002 FF/year.

Organization

Mlle Otz or Mme Burg
Agence du Médicament
Unité Contrôle National de Qualité
93285 Saint-Denis, France
Tel (33) 48 13 24 01. Fax (33) 48 12 23 56.

Analyte and matrix covered

Lead in total human blood.

Country

Federal Republic of Germany

Status of Program

National, but open to participants from other countries. Compulsory for German laboratories performing toxicological analyses in occupational medicine. Run by the German Society of Occupational and Environmental Medicine.

Aim: To fulfill the legal demands set by the technical guideline for quality control of toxicological analyses, as part of the code governing the handling of dangerous substances (TRGS410 GefStoffV); in addition to offer an intercomparison programme in the field of toxicology analyses in occupational and environmental medicine for research laboratories on a voluntary basis.

Participants: About 120 laboratories of all types performing toxicological analyses in occupational and environmental medicine.

Program description

Control materials: In-house preparations; human (urine) and animal matrix (whole blood, plasma); spiked concentrations; multielemental samples; liquid materials; glass vials for plasma and solvents in whole blood and urine; plastic vials for metals in whole blood and urine; assigned values are established in multiple analyses by several national and European reference laboratories.

Internal quality control samples/calibrators: No provision of this type of material; calibration and internal quality control has to be organised by each participating laboratory.

Organization of EQAS exercises: Frequency of exercises: a least one run per year; number of samples: two samples for each analyte and matrix; strategy of distribution: shipping by surface mail after registration of the laboratories for the current run; time schedule for returning results: within approximately five weeks after deadline of EQAS run; methods of transmission of results: by surface mail.

Elaboration of results: Preparation of a Youden-plot for each analyte and matrix and a certification report for each laboratory.

Criteria for evaluation of laboratory performance: Both results of a participating laboratory for a given parameter must lie within the acceptable range evaluated by the reference laboratories in order to obtain certification.

Measures taken against poor performers: No certification.

Provision of advice and training: Carried out frequently on the basis of personal communications and contacts.

Financial support: Registration fee of participants.

Organization

German Society of Occupational and Environmental Medicine
Professor Dr. Jürgen Angerer
Institut für Arbeits-u. Sozialmedizin und Poliklinik
für Berufskrankheiten der Universität, Schillerstraße 25-29
D-91054 Erlangen, Germany
Tel +49 9131-852374. Fax +49 9131-852317.

Analyses and matrices covered

Metals in whole blood and urine, fluoride in urine**.**
Aromatic and chlorinated hydrocarbons in whole blood.
Organochlorine compounds (e.g., DDE and HCH) polychlorinated biphenyls and pentachlorophenol in plasma. Metabolites of organic compounds in urine.

Country

Italy

Name of Program

METOS.

Status of Program

National but open to foreign participants; voluntary.
Run by Department of Clinical Biochemistry, National Institute of Health.

Aim: Continuous development and optimization of procedures for EQA; assessment of analytical performance provided by Italian laboratories; improvement of analytical performance and education of operators.

Participants: More than 100 laboratories, including local prevention units (51%), commercial (18%), hospital (15%), university and research laboratories (14%).

Program description

Control materials: Lyophilised, single-element samples (except for Cu/Zn) in plastic vials; prepared at the ISS from cow blood, cow/horse serum and human urine; different concentrations obtained by: addition of caeruloplasmin (serum Cu); pooling serum samples with high and low selenium content (serum Se); addition of known amounts of analytes (all other analytes); target value: median of the results of all laboratories or values obtained by the organisers in the preliminary analyses ("excepted values").

Internal quality control samples: Initially included in the programme but not more available to participants.

Organization of EQA exercises: EQA exercises organised every three/four months: for each analyte/matrix six to eight samples, including some as unknown duplicates, are sent to each participant. The samples assigned to each laboratory are randomly selected among the pools available and given reference codes using a computerised procedure to avoid identification and exchange of information. Results returned within 30 days by fax, post, or modem.

Elaboration of results: Median and parametric statistics for each sample. Linear regression between laboratory results (y) and target values (x).

Criteria for evaluation of laboratory performance: Rank of the laboratory within the group of participants (absolute and normalised rank); deviation from expected values, q-score and z-scores; deviation from the median and its comparison with established acceptability limits. Overall performances of laboratories evaluated once a year, from the percentage of acceptable results: 80 to 100%, "good performers"; 50 to 79%, "acceptable performers"; less than 50%, "poor performers."

Measures taken against poor performers: None.

Advice and training: Available on request.

Financial support: From the own resources of ISS; participation is free of charge.

Organization

Prof. Gino Morisi, Dr. Antonio Menditto, Dr. Marina Patriarca
Laboratorio di Biochimica Clinica, Istituto Superiore di Sanità
Viale Regina Elena, 299 - 00161 Roma, Italy
Tel + 39-6-49902559/31.
Fax + 39-6-4461961/8380.

Analytes and matrices covered

Aluminum, copper, selenium, zinc in serum.
Lead and cadmium in whole blood.
Chromium and nickel in urine.

Country

The Netherlands

Status of Program

Essentially national with some participants from other countries.

Participation on voluntary basis. Run by SKZL (Foundation for Quality Assessment in Clinical Laboratories) major.

Aim: Quality improvement.

Participation: 25 medical laboratories (clinical chemistry and toxicology laboratories).

Program description

Control sample: Prepared in laboratory from a human matrix (whole blood, serum and urine). Sample enrichment by addition of salts of trace elements. Multielemental, frozen samples in plastic vials. Dual target values: weighed amounts and mean of all laboratories.

Intended quality control: Spare samples from the EQAS available as check samples.

Organization of EQA exercises: Twelve samples distributed frozen once a year, one sample analysed every month. Results returned four times a year. Transmission by conventional post. Three provisional reports (every three months) and a final report (once a year) provided to participants.

Elaboration of results: Results elaborated after exclusion of outliers (data outside the interval: mean ±3 SD). The final report includes: evaluation of accuracy, in terms of recovery and proximity to the mean of all results; interlaboratory precision, linearity, evaluated by linear regression on the results provided in the year.

Criteria of evaluation of laboratory performance: No score system or certification; no criteria for evaluation of laboratory performance.

Measures against poor performers: None.

Advice: Given in annual meetings of participants.

Financial support: Costs covered by participation fee of laboratories.

Organization

C.W. Weykamp and T.J. Penders
SKZL, Section Multi Component Analysis
Beatrix Park I
NL-7101 BN Winterswijk, The Netherlands
Tel + 31 543 544774. Fax + 31 543 524265.

Analytes covered

Whole blood: Cd, Co, Hg, Se, Pb.
Serum: Co, Se, Mg, Li, Al, Cu, Zn.
Urine: Tl, Cd, Co, Hg, Pb, Mg, Cu, Zn, As.

Country **Spain**

Name of Program Programa interlaboratorios de control de calidad de plomo en sangre (PICC-PbS).

Status of Program International, voluntary.
Run by: Instituto Nacional de Seguridad e Higiene en el Trabajo (INSHT)

Aim: To provide laboratories with the means to: plan their quality assurance; detect trends; assess the performance of their analytical procedures.

Participants: 79 participating laboratories, of whom 53% Spanish, 4% Portuguese, and 43% from Latin-American countries (1994). The field activity of 58% of participants is occupational health. The remaining 42% includes hospital laboratories, universities, and other work activities related with health, toxicology, and environmental protection.

Program description **Control materials:** In-lab preparation. Human blood matrix. Each batch of samples is prepared from blood from the same donor, free from infectious-contagious factors, to which lead as an inorganic salt is added. Liquid, mono-elemental samples, in plastic vials. "Consensus Mean": calculated for each sample from the results falling within the ±2 SD from the overall mean ("accepted results"). To provide additional information, a "target value" is estimated for each sample. This is calculated taking the mean of the results from a group of laboratories that have achieved a good performance in the last three rounds (at least eight of the last nine samples have had results within 15% of the "consensus mean").

Organization of EQA exercises: Every month, each participant receives three samples, the lead content of which ranges from 20 to 90 µg/dL (0.965 to 4.343 µmol/L). Distribution: by mail. Time schedule for returning results: 30 days. Methods of transmission of results: communication by mail or by fax is maintained using a reference number (names of the participants are kept anonymous by the organizer) for each participant and their analytical method.

Elaboration of results: Each participant receives his results and overall picture of all results, histogram, along with the "consensus mean," the "target value," and their performance indices.

Criteria of evaluation of laboratory performance: Two performance indices are used. The variance index (IV) is calculated as the absolute percentage deviation (E) of the result returned by a participant (X) from the consensus mean (D) expressed as a percentage of a chosen coefficient of variation (CCV=15%), i.e.: IV = (E x 100)/CCV.E= ((X.D) x 100)/D. The mean variance index (IVM) is calculated as the mean of the last ten IVs. The IVM is updated every time a result is returned. Laboratories are considered to show: poor performance, if IV and IVM < 100; acceptable performance, if 60≤ IVM ≤ 100; a high level of performance, if IVM <60.

Measures taken against poor performance: None.

Provision of advice and training: None.

Financial support: INSHT resources. Participation is free of charge.

Organization

Daniel Marcuello
Gabineta Tecnico Provincial de Serguridad e Higene en el Trabajo-INSHT
C/Bernardino Ramazzini s/n, 50014 Zaragoza, Spain
Tel 76-516600.76-510639. Fax 76-510427.

Analytes and matrix covered

Blood lead.

Country

United Kingdom

Name of Program

Guildford trace elements external quality assessment scheme (TEQAS).

Status of Program

International, national (UK NEQAS), voluntary.
Operated by the Centre for Clinical Sciences School of Biological Sciences, Guildford, Surrey, GU2 5XH

Aim: Educational and to stimulate improvements in analytical performance.

Participants: 172 (70 within UK and 102 in 25 other countries). Hospital laboratories: 108; private clinical laboratories: 23; occupational health departments: 19; university and veterinary laboratories: 14; industrial organizations: 6; government facilities: 2.

Program description[4,5]

Control materials: Prepared in the laboratory from bovine/equine serum, human whole blood, and urine. Addition of known amounts of analytes to liquid pools. Liquid, multielemental samples, in plastic vials. Target value chosen as the consensus median of laboratories' results after exclusion of outliers (i.e., results falling outside the range mean ±3 SD). Internal quality control samples or calibrators are not distributed as formal component of the scheme. Some RMs are manufactured for participants to use as routine IQC samples.

Organization of EQA exercises: Monthly distribution with three specimens per month, within a six-month cycle. Samples are sent at beginning of month, results should be returned by last day of month by fax, post, or telephone.

Elaboration of results: Computer program to calculate means, medians, standard deviations, coefficients of variation, and histogram displays of the distribution of results.

Criteria for evaluation of laboratory performance: a) comparison of laboratory results at the end of each six-month cycle with target zones for proximities to assigned values, difference between results for duplicate measurements, and recovery of added analyte; b) monthly and cumulative scores derived from proximity of results to be the assigned values (equivalent to "z-scores"). Standards for acceptable and unacceptable performance have been developed.

Measures taken against poor performers: a) all laboratories are informed with the monthly reports as to whether current performance is acceptable or unacceptable; b) for UK NEQAS participants only, if poor performance continues, a personal letter is sent to the head of department to encourage corrective action and to offer advice and support. Where problems persist, the situation is reported (anonymously) to the National Quality Assurance Advisory Panel for Chemical Pathology.

Advice, assistance and training: Offered to poor performers (see above), and previously assayed samples are made available to help investigate problems. Personnel visit other laboratories in the UK and abroad for ad hoc teaching, training, and problem-solving purposes, but there is no formal training at this time.

Financial Support: From participant subscriptions.

Organization

Centre for Clinical Sciences, School of Biological Sciences, Guildford, Surrey GU2 5XH
Dr. Andrew Taylor
Tel + 44 1483 879217.
Fax + 44 1483 879979.
e-mail A.Taylor@surrey.ac.uk

Analytes and matrices covered

Established
Serum: aluminum, copper, gold, selenium, zinc.
Whole blood: lead, cadmium.
Urine: mercury, cadmium.
Dialysis fluid, water: aluminum.

Under development
Whole blood, urine: arsenic, chromium, copper, cobalt, iron, magnesium, manganese, nickel, zinc.

Country **United Kingdom**

Organization

UK NEQAS for Lead & Cadmium in Blood
Wolfson EQA Laboratory
PO Box 3909
Birmingham BI5 2UE
United Kingdom
Contact person: Dr. David Bullock
Tel 44 (0)121-414 7300. Fax 44 (0)121-414 1179.
e-mail: queries@ukneqas.org.uk
Website: www.ukneqas.org.uk

Status of Program

National, but open to foreign participants; voluntary. UK National External Quality Assessment Scheme. Participation in this scheme is compulsory for UK laboratories carrying out measurements for occupational exposure to lead in accordance with the UK "Control of Lead at Work Regulations 1988."

Aim: Educational and to promote improvement of laboratory performance for blood lead.

Accreditation: Unconditional accreditation awarded by Clinical Pathology Accreditation (UK) Ltd, September 1997; reinspected September 1999.

Participants: 83

Program description[6]

PT materials: Liquid sonicated whole blood hemolysate, prepared from fresh equine blood preserved with EDTA; target values are the all-method mean of participant laboratories.

Organization of PT events: Samples are sent twelve times per annum, two specimens per batch. A web-based service is available for results submission and report retrieval.

Elaboration of results: Reports include a frequency distribution graph, summary statistics, and two performance progress graphs for the participant laboratory showing the Mean Running Variance Index Score (MRVIS) and the Mean Running Bias Index Score (MRBIS); a revised scoring system including compensation for concentration-dependence of performance is in development.

Criteria for evaluation of laboratory performance: Criteria are based on attainable and desirable performance, currently an MRVIS[6] of 60, representing an average percentage deviation (ignoring sign) from target of 9.0%; results must be returned for at least 75% of specimens.

Measures taken against poor performance: The primary objective is educational, but laboratories outside criteria and providing a clinical service in the UK are contacted initially by the Scheme Organiser to provide advice and assistance. Failure to respond or improve may entail referral to the National Quality Assurance Advisory Panel for Chemical Pathology and/or prohibition from providing a biological monitoring service under the Lead at Work Regulations.

Financial support: Self-financing scheme, operated in the public sector and supported by participant laboratories' subscriptions.

References to Appendix B

1. Weber JP. Quality in environmental toxicology measurements. *Therapeut Drug Monit.* 1996;18:477-483.

2. Parsons PJ. Monitoring human exposure to lead: an assessment of current laboratory performance for the determination of blood lead. *Environ Res.* 1992;57(2):149-162.

3. Stanton NV. Blood lead proficiency testing: overview of the federally sponsored program in the U.S. *J Int Fed Clin Chem.* 1998;5:158-161.

4. Taylor A, Briggs RJ. An external quality assessment scheme for trace elements in biological fluids. *J Anal At Spectrosc.* 1986;1:391-395.

5. Taylor A, Briggs RJ. The Guildford trace elements external quality assessment scheme. *Ann 1st Super Sanità.* 1986;32(2):253-259.

6. Bullock DG, Smith NJ, Whitehead TP. External quality assessment of assays of lead in blood. *Clin Chem.* 1986;32:1884-1889.

Appendix C. Reporting Practices, Risk Classifications, and Rescreening Timetables Recommended for Blood Lead Results in the United States

C1 Electronic Reporting Policies for U.S. Blood Lead Laboratories

After the determination of the patient's BPb level, U.S. laboratories are requested (or in some states required) to report either elevated or all BPb results to the state in which the patient resides. This reporting is accomplished in either electronic form (e.g., direct transfer by modem, Internet, or possibly diskette) or a paper transfer. The preferred mode of transfer is electronic due to elimination of potential data entry errors during data transcription. Many states have adopted a common database structure for reporting BPb test results electronically.

The table below shows the consensus of the CDC-funded childhood lead surveillance grantees and other state childhood lead surveillance groups list of childhood lead reporting variables.

Childhood Lead Reporting Variables

Patient's First Name
Patient's Middle Name
Patient's Last Name
Address 1 (Street 1)
Address 2 (Street 2)
City
State
Zip
County
Parent's First Name
Parent's Last Name
Phone Number
Patient's Date of Birth
Gender
Race
Hispanic (Yes, No)
Patient's Medicaid Number
Healthcare Provider (person that collected the blood sample)
Healthcare Provider (Name of the practice, HMO, hospital, or clinic)
Healthcare Provider Phone Number
Healthcare Provider Street Address
Healthcare Provider City
Healthcare Provider State
Healthcare Provider Zip
Date Sample Collected
CLIA Laboratory ID Number
Change to Previously Submitted Report
Laboratory Accession Number
Laboratory Requisition Number
Date of Analysis
Metal Tested (e.g., lead, mercury, cadmium, arsenic)
Type of sample (e.g., venous blood, fingerstick blood, urine)
Purpose of test (e.g., screening, clinical suspicion of poisoning, confirmation, follow-up, EP, etc…)
Test Result
Units of test result
Comments

State surveillance systems are based on reports of BPb tests from laboratories. Ideally, laboratories report results of all BPb tests, not just elevated values, to the state health department. However, it is up to the states to determine the reporting level for BPb tests and to decide which data elements should accompany the BPb test result. To assure identification of duplicate test results and sequential tests on a single child, BPb test results are maintained by the state in a child-specific database. In addition to BPb test results, state child-specific databases contain follow-up data on children with elevated BPb levels, including data on medical treatment, environmental investigations, and potential sources of lead exposure. Surveillance fields for the national database are extracted from the state child-specific database and transferred to CDC.

CDC began surveillance of BPb levels among children in 1992. The goals of the national childhood lead surveillance program are to establish childhood lead surveillance systems at the state and national levels and to use surveillance data to estimate the extent of elevated BPb levels among children, assess the follow-up of children with elevated BPb levels, examine potential sources of lead exposure, and help allocate resources for lead-poisoning prevention activities.

Steps in a complete surveillance system for BPb levels among children include:

(1) **Transfer of data from laboratories to the state health department**
Both public and private laboratories transfer data to state health departments. Laboratories that process a large number of samples are asked to submit data via modem or diskette. To preserve confidentiality of patient data, files are encrypted and compressed before being transmitted.

(2) **Transfer of data from the state health department to local health departments**
States transfer laboratory data to local health departments responsible for follow-up of children with elevated blood-lead levels. Data are transmitted electronically or via paper-based systems. In a few states, transfer of data from state to local health departments is unnecessary, as the state is responsible for follow-up of children with elevated BPb levels.

(3) **Transfer of data from local health departments to the state health department**
Follow-up data on children with elevated blood-lead levels collected by the local health department include demographic data, potential sources of lead exposure, environmental investigation data, and lead-hazard remediation data. The data are usually maintained in a computerized database at the local level. Surveillance data fields are extracted from this database and sent to the state health department. At the state level, data from the local health departments are collected in a single surveillance database.

(4) **Transfer of data from state health departments to CDC**
The state submits the surveillance database, without patient identifiers, to CDC. Data transfer is electronic, usually through PC-WONDER. Data transfer from state health departments to CDC is planned quarterly.

(5) **Transfer of data from CDC to state health departments**
After performing data quality checks, CDC will return the surveillance data to the state health department. CDC is developing ways to facilitate data analysis at the state level.

C2 Comprehensive Follow-up Services, According to Diagnostic Blood Lead Level. Reproduced with permission from Centers for Disease Control and Prevention. *Screening Young Children for Lead Poisoning: Guidance for State and Local Public Health Officials*. Atlanta: CDC; 1997.

BLL (μg/dL)	Action
<10	Reassess or rescreen in one year. No additional action necessary unless exposure sources change.
10-14	Provide family lead education. Provide follow-up testing. Refer for social services, if necessary.
15 -19	Provide family lead education. Provide follow-up testing. Refer for social services, if necessary. If blood-lead levels persist (i.e., two venous blood-lead levels in this range at least three months apart) or worsen, proceed according to actions for blood-lead levels 20-44.
20-44	Provide coordination of care (case management). Provide clinical management. Provide environmental investigation. Provide lead-hazard control.
45-69	Within 48 hours, begin coordination of care (case management), clinical management, environmental investigation, and lead-hazard control.
≥ 70	Hospitalize child and begin medical treatment immediately. Begin coordination of care (case management), clinical management, environmental investigation, and lead-hazard control immediately.

C3 Schedule for Diagnostic Testing of a Child with an Elevated Blood Lead Level on a Screening Test. Reproduced with permission from Centers for Disease Control and Prevention. *Screening Young Children for Lead Poisoning: Guidance for State and Local Public Health Officials*. Atlanta: CDC; 1997.

If result of screening test (μg/dL) is:	Perform diagnostic test on venous blood within:
10-19	3 months
20-44	1 month - 1 week[7]
45-59	48 hours
60-69	24 hours
≥70	Immediately as an emergency laboratory test

[*] The higher the screening blood-lead level, the more urgent the need for a diagnostic test.

NCCLS consensus procedures include an appeals process that is described in detail in Section 9.0 of the Administrative Procedures. For further information contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Subcommittee Responses

C40-P: *Analytical Procedures for the Determination of Lead in Blood and Urine; Proposed Guideline*

General

1. In general we feel the proposed guideline is too long. It is very repetitive on many issues. Often certain matters are repeated several times in different sections and these could be merged. Better use of other NCCLS guidelines/standards as references would eliminate the requirement to describe certain procedures that are best addressed in these documents.

- **The document is deliberately repetitive on the issue of quality assurance/quality control (QA/QC) because of the need to reinforce method-specific QA/QC recommendations that logically belong alongside descriptions of those procedures, and yet might otherwise be overlooked. The subcommittee believes that those aspects of QA/QC which are independent of analytical method are better grouped into a single section. Numerous cross-references are provided to assist the reader. As for using other NCCLS guidelines/standards instead of describing certain (presumably collection) procedures, the subcommittee feels very strongly that generic procedures for blood/urine collection that are available in other documents do not adequately address the special requirements and considerations for determining lead, a ubiquitous element, and especially the need to minimize contamination errors. The subcommittee also feels that providing detailed instructions that take special account of contamination concerns is more helpful to the end user and makes a more comprehensive document.**

2. The document title does not reflect the content. For example, Sections 4.1, 4.2, 4.2.1, and 4.2.2 contain information which is available elsewhere and unduly increases the length of this document if the purpose is to provide analytical procedures. I would suggest that either the title be changed to broaden the scope or the document drastically reduced to focus on the intended subject.

- **The subcommittee believes that this document should represent nothing less than a comprehensive treatment of the measurement of lead in blood and urine that will serve as a useful bench guide as well as an educational resource. We elected to include a section on the clinical significance of lead measurements to help laboratorians and others understand the context in which these measurements are made. We believe the document is much improved as a result. While we have modified the foreword and the scope description to reflect the comprehensive nature of the document, we continue to believe that a simple title is appropriate and should not be changed.**

3. The subcommittee should reevaluate which international symbols would be most appropriate to represent blood lead and urine lead.

- **Because there are no internationally accepted symbols for blood lead or urine lead, the subcommittee elected to combine the chemical symbol for lead with a letter representing the specimen. This approach has been adopted in many published papers on lead poisoning.**

Foreword

4. Last sentence of the second to last paragraph: "As a relatively high, cost, multielement technique, ICP/MS is unlikely to be used for routine BPb measurements." Currently there are 22 labs participating in the ICP/MS comparison program conducted by Lacentra Detoxicology du Quebec. The number of labs using ICP/MS to analyze trace metals including lead is increasing. Cost-efficiency for any method or instrument is a function of several variables including testing volume, labor costs, run frequency etc. It is inappropriate for an emerging, high-quality technique to be dismissed by the committee because of their commitment to atomic absorption technology.

- **The subcommittee respectfully disagrees that it has dismissed ICP-MS because of its commitment to atomic absorption technology. The comment is especially surprising given that this subcommittee includes members who have been among the early pioneers in publishing clinical applications of ICP-MS instrumentation, including methods for BPb, and those responsible for introducing the technology commercially in the U.S. The valuable contribution that ICP-MS has made in areas such as Pb isotope ratio measurements for source identification and, in isotope dilution mode, as a fundamental reference technique for BPb are fully described and referenced in Section 5.6. At the time when the proposed document was written, the cost of ICP-MS instrumentation was much greater than that of ETAAS at least by a factor of five or more. For most routine laboratories, where BPb may be the only trace element measured, ICP-MS instrumentation would clearly not be a cost-effective choice. As the commentor states, cost-efficiency is a function of several variables, including testing volume, labor costs, run frequency etc., and we would certainly agree with this. The ICP-MS program offered by Le Centre de Toxicologie du Québec (CTQ) is an international comparison program for up to 23 trace elements in serum, whole blood, and urine (Appendix B4). We note that, in the BPb program offered by CTQ, only 5 out of 100 participants use ICP-MS, while in U.S.-based proficiency testing programs for BPb, ICP-MS accounts for less than 4% in the New York State program, and <2% in the Wisconsin PT program. In the UK NEQAS for BPb, <2% of participants use ICP-MS. So, at the current time, ICP-MS is limited to a minority of BPb laboratories, but this fact should not be construed as dismissive. It may well be that future revisions of this document could include recommended procedures for BPb by ICP-MS as the technique becomes more cost-effective and more laboratories gain experience.**

Section 5.6.1

5. Third paragraph, first sentence: Laboratories have reported ICP/MS instrumentation to analyze blood lead samples. In our laboratory, we are analyzing from 400 to 500 blood lead samples daily using ICP/MS. The last paragraph states, "However, it is doubtful that ICP/MS will ever be an important method for routine monitoring of blood Pb." This negative slant to ICP/MS is inappropriate as it is an emerging technology which has considerable advantage over absorption methods.

- **The statement in Section 5.6.1, third paragraph, first sentence has been modified to reflect that a minority of laboratories are analyzing large numbers of routine patient samples for blood lead by ICP-MS. Again, the subcommittee did not intend for its statements to be interpreted as a "negative slant" toward ICP-MS. Thus, we have deleted the final sentence from the last paragraph in Section 5.6.1 and have added the following reference that describes a routine ICP-MS method for BPb:**

  "Nuttall KL, Gordon WH, Ash KO. Inductively coupled plasma mass spectrometry for trace element analysis in the clinical laboratory. *Ann Clin Lab Sci.* 1995;25:264-271."

Section 6.1.1

6. I take issue with the statement: "The use of filter paper for lead determination is currently controversial,…procedures," for the following reasons: (The statement should be rewritten or deleted, as it attempts to create doubt about the analytical chemistry quality of FP/Pb testing.)

(a) The citation of a letter to the editor of *Clinical Chemistry* offers no scientific data or findings to back the assertions made concerning the suitability of FP/Pb testing. This letter is not scientific proof that the preanalytical problems are unique or generic for FP/Pb testing or that the method does not work for the purpose described.

(b) The preanalytical concerns are ones that any competent analyst must deal with in the performance of trace metal measurements. There is no need to insinuate that they are unique or apply only to FP/Pb testing.

(c) No mention is made of several recent publications in peer-reviewed scientific journals describing successful uses of FP/Pb testing.

(d) I believe FP/Pb testing can be utilized successfully for lead testing. For underdeveloped countries, the low cost of collection materials, relative ease of obtaining blood from young children, stability of FP blood samples, and FP samples can be mailed to a distant laboratory for testing are all attractive advantages to be considered for a lead-screening program.

- **The text has been revised to include literature citations, both articles and letters, that address the FP/Pb measurement process.**

7. (a) Many times we used to receive filter papers holding wet blood inside plastic sleeves... the dried blood spots usually do not show a uniform color, and it is often difficult to expect reproducibility. These blood lead filter papers were analyzed using flame atomic absorption spectrophotometry, and the smoke and lead peaks were not as well defined as with whole blood.

(b) It is now many years since I used the technique of dried blood spots on filter paper and the Delves cup procedure... The filter paper procedure proved problematic in terms of absorption of blood into the filter paper but specifically in the problems of contamination. Lead is ubiquitous in many laboratory materials and indeed in the laboratory air. Due to widespread use of leaded gasoline significant aerial depositions of lead occurs. Therefore, it is essential that samples for analysis are not exposed to laboratory air and that an additional contaminating matrix is not present in the analytical step. These reasons have lead to the discontinuance of the use of filter paper for blood specimen collection. Such procedures are now widely acknowledged as being inappropriate in laboratories in developed countries.

(c) Over 15 years ago, the filter paper method was not recommended for environmental monitoring in a European survey due to concerns over contamination risks. Although lead usage has declined dramatically over this period, lead is still a ubiquitous contaminant in dusts both in urban and suburban areas, and the concern over contamination is still valid, particularly so for the measurements at the 10-µg/dL level or below. Risks of contamination are minimized by use of well-characterised sampling materials and minimal sample manipulation during collection and analysis. In the case of filter paper methods, there is clearly more sample manipulation than for liquid sampling methods, and therefore increased risk for contamination.

(d) Our experience of trace analysis issues supports the view that the use of an absorbing medium, followed by "solid sampling" (i.e., a punched disc of filter paper), is bound to complicate and degrade to some extent the analytical process.

- **The text has been revised to include literature citations, both articles and letters, that address the FP/Pb measurement process.**

8. Background, paragraph 3, first sentence: In our experience, and in review of the most common commercially available capillary collectors, typical fill volumes are between 200 µL and 750 µL. Some systems do offer options that are prepared for the collection of as little as 100 µL or as high as 1mL (1,000 µL) of blood sample. Therefore, we recommend the first sentence to be revised to reflect the varied range of products available as follows; *Several types of plastic microcollection containers (typical fill volumes range from 100 µL to 1 mL are available for…"*

- **The text has been modified as suggested.**

Section 6.1.2

9. The sixth bullet states that EDTA is normally preferred over heparin. Since EDTA is a chelating agent that will readily adsorb lead from the environment, and heparin is not, it would appear that heparin should be the preferred anticoagulant. It would be interesting to know what significant disadvantages exist for heparin for lead testing that outweigh this significant disadvantage for EDTA. Usually, commercially produced capillary collection products contain sufficient heparin to anticoagulate a sample and keep it in satisfactory condition for testing for up to seven days. This should be enough time, even in the most adverse situations, to transport and test a sample. Our own search through textbooks referring to lead analysis has only found references to heparin as the anticoagulant of choice. For example: David S. Jacobs et al. *Laboratory Test Handbook.* 2nd ed. ISBN 0-683-04368-4. We recommend that this statement be given more consideration.

- **The document states that either heparin or EDTA anticoagulants are acceptable, but that EDTA is normally preferred. The primary rationale for the preference of EDTA, the absence of microclot formation, is described in Section 10.3.2. The reviewer's argument that, as a chelating agent, EDTA will readily absorb lead from the environment, would suggest contamination of the salt during the tube manufacturing process is a problem. However, the experience of the subcommittee members who have tested different batches of blood collection tubes over the last decade indicates that lead contamination of EDTA tubes is not a significant problem. On the contrary, microclot formation in heparinized blood is a much more serious problem that inevitably leads to specimen rejection.**

10. Eighth Bullet: In our opinion this bullet should be revised to include appropriate comment for the sharps containers. We recommend the following: *"Bags and containers for medical waste and sharps respectively should be clearly identified as such."*

- **The bullet has been modified to include the comment for identifying the sharps container: *"Bags and containers for medical waste and sharps, respectively, should be clearly identified as such."***

11. Last paragraph, first sentence: Even if manufacturing is carefully controlled and prepared to reduce lead contamination, it is virtually impossible for the final product to be lead-free. Therefore, we recommend this sentence be revised as follows: *"Material used in the collection procedure that could contaminate the specimen (for example, blood containers and alcohol swabs) must be free from significant lead contamination."* A definition for "significant lead contamination" should be developed and added to an appropriate section.

- **We accept the reviewer's suggestion. We have substituted: "*free from significant lead contamination"* for lead-free, and have added: "*(see Appendix A for guidance on contamination***

testing and Section A1.9 for interpretation of results and definition for "significant lead contamination)."

Section 6.1.2.2

12. In order to ensure there is no misunderstanding, about when massaging a finger should occur to increase circulation, we recommend the following revision for paragraph 3: "*If desired, a brush can be used for cleaning the finger. Brushing or massaging the finger during washing can increase circulation in preparation for puncture with the lancet."* Paragraph 4 should also be revised to read: *"The finger to be punctured (often the middle finger) must be free of any visible infection or wound."*

- **The procedure given here is consistent with the references cited and with current public health practice. Massaging the finger can be accomplished either during or after the washing step. The wording has been modified to ensure there is no misunderstanding:**

  "...the finger must not be allowed to come into contact with any surface, (e.g., door, chair, counter-top, or even the child's other fingers), except the gloved hand of the person performing the procedure."

  The aim is that, once washed, the clean finger is not recontaminated by touching surfaces which may be contaminated. Provided the person performing the fingerstick procedure is wearing clean, sterile latex/vinyl examination gloves, no recontamination should occur.

Section 6.1.2.3

13. Procedure for Preparing the Child's Finger: Point (4) should be eliminated. This practice encourages lead contamination and adequate massaging of the finger can occur during the washing stage.

- **The subcommittee disagrees with this statement. See our response to Comment 12.**

Section 6.1.3

14. Filling the Collection Container: Paragraph 1, line 9 states that "...it is more important to prevent blood clotting during transit to the laboratory than cell rupture." Specifying "during transit" only is incorrect. Preventing the blood from clotting at all stages prior to analysis is more accurate. We recommend this sentence is revised as follow: *...it is more important to prevent blood clotting prior to analysis than cell rupture."*

- **We have reworded the last sentence of paragraph 1 as follows: *"This is not an issue for BPb, since the prevention of clotting prior to analysis is more important than preserving erythrocyte integrity."***

Section 6.2.1

15. Required Supplies, first bullet: There are blood collection systems available that can collect a venous blood sample using an evacuated method, a syringe principle of collection or normal venous pressure. These systems are efficient and produce high-quality samples. Based on availability of these systems, bullet 1 should be changed to read; *"Prescreened EDTA blood-collection tubes (0.5 mL or greater)."*

- **The reviewer is correct: commercial blood collection systems exist that comprise a combined capillary and venous blood collection device consisting of a removable Luer-lock needle attached to a 600-µL microcontainer, and are available with potassium EDTA anticoagulant. Although the subcommittee members have no personal experience with such devices, if they**

function properly and background lead contamination is minimal, then they should be suitable for collection of venous blood volumes <1.0 mL for blood lead determination. Accordingly, this sentence has been reworded as suggested: *"Prescreened EDTA blood-collection tubes (0.5 mL or greater)."*

16. Is a 2- to 3-mL sample absolutely necessary for lead testing? In our experience, 0.5 mL is sufficient sample to carry out the test required. There are some venous blood collection products available that can collect as little as 0.6 mL of sample. These should not be unnecessarily excluded from the guideline.

- **As stated above, the sentence has been reworded as suggested to accommodate new commercial blood collection devices designed to collect <1.0 mL venous blood.**

17. Bullet 3 states, "Needle/tube holders." There are blood collection systems that do not require the use of needle/tube holders. Therefore, bullet 1 should be changed to read; *"Other accessories, as required"* or *"Needle/tube holders if required."*

- **The sentence has been reworded as suggested: *"Needle/tube holders if required."***

Section 6.2.2

18. Preparation: To ensure consistency with the change recommended in 6.2.1 Required Supplies, bullet 1, lines 1 and 2 should be revised to read as follows; *"bandages, blood specimen tubes and accessories (as required), tourniquets, labels…"*

- **The sentence has been reworded as suggested: *"Assemble all required blood collection and approved needle-disposal materials in advance (see Section 6.2.1, Required Supplies). Wear disposal latex or vinyl gloves, laboratory coat, and eye protection."***

19. Why repeat everything that has already been specified in the preceding Section 6.2.1 Required Supplies? A simple statement as follows would be sufficient; *"Assemble the required blood collection materials (see 6.2.1 Required Supplies)."*

- **See response to Comment 18.**

Section 6.2.3

20. Blood Collection: Paragraph 1, line 7 should be revised to ensure consistency with the change recommended in 6.2.1 Required Supplies, bullet 1. The sentence should be revised as follows; *"…'butterfly' needle in combination with 0.5 mL or greater EDTA specimen collection tube…"*

    We have eliminated the words "(purple-top)" from this sentence also. If this guideline is to gain international recognition, the color code cannot be specified here. In different parts of the world the color code for EDTA is different. For example, in Germany it is red.

- **The sentence has been revised as suggested: *"Collect blood using either a regular multisample needle or a "butterfly" needle in combination with a 0.5-mL or greater EDTA specimen collection tube, filling the tube as completely as possible."* All references to container cap color have been removed.**

21. Paragraph 1, lines 8 and 9 reference a Note to new plastic tubes and varying nominal volumes. Some glass evacuated tubes are the same physical size but differ in the amount of sample that can be

collected depending on the vacuum contained. This comment should not be specific to plastic containers, has no relevance here and should be eliminated.

- **The intention of this statement is to make the phlebotomist aware that while certain evacuated collection tubes may be the same physical size, the draw volumes can be variable. The reviewer is correct that both plastic and glass evacuated tubes are marketed with variable draw volumes. The sentence has been modified: *(NOTE: Some specimen tubes may be the same physical size with varying nominal volumes.)* The subcommittee feels this information is relevant and the phlebotomist should verify intended tube volume before use.**

22. Paragraph 2, line 3 states that the arm should be "held straight and elevated over the head for the several minutes." Elevating the arm over the head is not a common practice and is not consistent with other NCCLS guidelines and standards. The words "and elevated over the head" should be removed.

- **The purpose of this sentence was to emphasize that the subject's arm should not be bent at the elbow after phlebotomy, which may induce the formation of a hematoma. However, this section has been rephrased to be consistent with NCCLS document H3— *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture.***

Section 6.3.1

23. 24-Hour collection, Point (1) references a footnote "a": Since it is virtually impossible for containers to be free from lead contamination, we recommend that the footnote is revised as follows: *"Lead-free is interpreted as either (a) acid-leached/washed, or (b) lot-tested to ensure that the containers are free from significant lead contamination."*

    As stated above, it may be prudent to define what "significant lead contamination" means. Also, is the fact that a container has been acid-leached washed sufficient to guarantee the contamination level is less than significant or should these be lot tested also?

- **We accept the reviewer's point. The text has been modified as suggested, but with some additional text in parentheses: *(see Appendix A for guidance on contamination testing and Section A1.9 for interpretation of results and definition for "significant lead contamination").* Routine acid washing of laboratory ware/containers (typically soaking with 1 to 2% v/v HNO_3 for 24 hours) is considered good laboratory practice, and is universally practiced in laboratories specializing in trace element analysis. Such practices certainly minimize contamination errors but can no more guarantee the absence of significant contamination than can random lot testing of laboratory tubes/containers.**

24. Point (2) refers to trace-element-free measuring cylinders. This should be revised to *"lead-free 2 L measuring cylinders"* to ensure the "a" footnote is relevant.

- **The text has been amended as suggested.**

Section 6.3.3

25. Eight-hour Timed Collection: Paragraph 2, line 3 references a footnote "b." This is exactly the same as the footnote "a" from the previous page 18. Why not reference this footnote as "a" also to avoid confusion.

- **We have deleted Footnote b.**

Section 7.5.4

26. Are there any guidelines for the acceptable slope range of calibration line? (Recording the slope without developing criteria for evaluating the calibration curve is not useful.)

- **The issue in question is the slope of the calibration line, which is instrument-dependent, and is an index of sensitivity, i.e., characteristic mass (m_o), along with the lamp energy. Criteria for evaluating sensitivity changes are provided in some detail in Section 7.7. We have added a cross-reference to Section 7.7 along with a sentence: "These data are useful for troubleshooting if quality control problems arise. See Section 7.7."**

27. I find the use of "seconds" as units for peak area confusing. It is most normally expressed as "Abs.s." I know that theoretically absorbance is a ratio and therefore dimensionless, but the use of "Abs.s" expresses better where the measurement comes from, whereas the expression of area as a time unit "seconds" is misleading.

- **Our use of "seconds" as units for peak area or "integrated absorbance" is consistent with recommendations of the International Union of Pure and Applied Chemistry (IUPAC) regarding quantities, units, and symbols and their use in physical chemistry, and with current recommendations in analytical atomic spectrometry.[9] According to IUPAC, a clear distinction should be drawn between the names and symbols for physical quantities and the names and symbols for units. In order to avoid confusion, symbols for physical quantities and units should not be combined. Thus the unit for the integrated absorbance is simply s (seconds) precisely because, as the commentor points out, absorbance is dimensionless.**

Section 9.2.3

28. Quality Control Samples; paragraph 1: Delete the second half of the last sentence, "and that a large ... material is stable."

- **We prefer to leave the statement in place because of the importance of continuity in internal QA/QC programs. Some laboratories buy only small quantities of material and then lose the opportunity of a comparison over a longer time period.**

29. Quality Control Samples; paragraph 4: I suggest the following revisions: "If commercial materials are used, a large enough quality from the same batch should be obtained to satisfy the requirements over the period that the material is stable. …… This QC material is then stored as recommended by the manufacturer for use later in the routine analysis with each batch of patient samples in a format discussed below."

- **The subcommittee believes it is important that where QC materials are either purchased commercially or prepared in-house, they should all be from the same batch and should be homogeneous among the several containers supplied. We do accept that, where commercial materials are used, they should be stored as recommended by the manufacturer.**

Section 10.3.2

30. Acceptable Specimen: Paragraph 1 states that "The preferred specimen for the determination of lead is whole blood with EDTA (1.5 mg/mL) anticoagulant." Again, we question if EDTA should be preferred over heparin. In our experience, heparin provides a good quality sample for testing for up to seven days. Also, most manufacturers of blood collection products state that the concentration of the dipotassium and tripotassium salts of EDTA shall be within the range 1.2 mg to 2 mg of

anhydrous EDTA per 1 mL of blood [EDTA is calculated as the anhydrous salt of sequestric acid (0.00411 mol/L to 0.006843 mol/L)]. This sentence should be revised to take this into consideration. In our experience lead tests are not affected within this range.

- **The preference of EDTA over heparin is addressed in our response to Comment 9. We accept that EDTA concentrations are only nominally 1.5 mg/mL blood and so the text has been modified accordingly. As noted in Section 10.3.4, bullet 6, excessive EDTA concentrations can cause a negative bias with anodic stripping voltammetry techniques.**

31. Paragraph 2 states that "Acceptable containers for BPb analyses include 2 to 7 mL evacuated blood collection tubes, [e.g., …" To ensure consistency with the various recommendations made above, this entire paragraph should be revised as follows; *"Acceptable containers for BPb analyses include venous blood collection tubes greater than 0.5 mL or plastic microcollection tubes greater than 100 µL in volume. Containers must be free from significant lead contamination."*

    We recommend removing all reference to color codes to ensure international acceptance of the guideline.

- **Consistent with previous revisions to accommodate commercial devices for collecting smaller-volume venous blood specimens, this sentence has been revised as suggested. All references to container cap color have also been removed.**

32. Paragraph 3 states that 50 µL of blood is required for a single test. Why, therefore, is the optimum volume of venous blood required stated to be 2 to 3 mL? This is a contradiction. As stated above, there are venous blood collection products, which do not utilize a vacuum, that can collect as little as 600 µL (0.6 mL) of blood and provide a high-quality sample. These should not be excluded from the guideline. This whole paragraph should be reviewed further and revised. It should also include the required volume of blood for diagnostic or medical purposes.

- **Here we distinguish between the minimum blood volume obtained from the subject, and the minimum aliquot of blood that is then removed for the analysis. The former is dependent on the specific commercial blood collection device used. We accept that commercial devices are now available for collecting as little as 600 µL of venous blood and the first sentence of paragraph 3 has been modified to*: "At least 500 µL of venous blood should be collected for diagnostic purposes."* The second sentence has been deleted. (See response to Comment 1.) However, the volume of blood removed for a single analysis will depend on the analytical method such that with most furnace AAS methods, it is typically as little as 50 µL. Of course a sufficient blood volume (capillary or venous) must be collected from the patient to ensure a representative sample is obtained, and to enable repeated testing based on the laboratory's IQC policies and routine method.**

Section 10.3.3

33. Specimen Stability: Paragraph 4 references a potential dilution effect of liquid EDTA. The volume of liquid EDTA in most commercially produced products is minimal compared with the volume of sample collected. Even if a tube is only half filled, the dilution effect is often less than 2%. Would this affect blood lead measurements significantly and therefore, is it necessary to recommend such a strict volume of collection? In our experience, capillary microcollection tubes are rarely filled to their correct volume, especially those with a fill volume greater than 500 µL. We recommend filling the blood collection tube greater than half its recommended fill volume.

- **With all collection tubes (micro- or evacuated), it is indeed critical that they are filled to at least half of their nominal draw volume in order not to over-dilute blood with liquid EDTA. Our statement was directed at situations where only 200 to 300 µL of blood are collected in a 2- or 3-mL nominal drawtube. In such situations, a low bias has been observed due to over-dilution effects from liquid EDTA. Full recoveries are not attained until these tubes are at least half-full (personal communication, Dr. Robert Jones, CDC, 1999).**

Appendix B

34. In Section B4, please add "Analytes and Matrix covered" as described in Section B5.

- **We have added a section on "Analytes and Matrices covered" to PT programs listed in Section B4 as requested.**

35. Here it may be helpful to readers to add a reference on page 69: Bullock DG, Smith NJ, Whitehead TP. External quality assessment of assays of lead in blood. *Clin Chem.* 1986;32:1884-1889.

- **The citation has been added to the references listed for Appendix B.**

36. The UK NEQAS for Lead and Cadmium in blood. It is not indicated that participation in this scheme is compulsory for laboratories carrying out measurements for occupational exposure to lead in accordance with the UK "Control of Lead at Work Regulations, 1988." Recent changes (1999) in this scheme have involved a switch from bovine blood to equine blood and a distribution of 2 specimens per batch 12 times a year, rather than 1 specimen 24 times a year.

- **This information has been added to the description of the scheme.**

37. Ray Briggs is no longer with the Guildford scheme, and the phone, etc. contact details on page 68 have changed.

- **Details for the Guildford trace elements scheme have been updated to reflect its reorganization under the United Kingdom's National External Quality Assessment Scheme (NEQAS) for Trace Elements.**

References to Summary of Comments and Subcommittee Responses

1. Verebey K, Rosen JF, Schonfeld DJ, et al. Blood collection and analytical considerations in blood lead screening in children. *Clin Chem*. 1995;41(3)**:**469-470.

2. Schonfeld DJ, Cullen MR, Rainey PM, et al. Screening for lead poisoning in an urban pediatric clinic using samples obtained by fingerstick. *Pediatrics*. 1994;94(2 Pt 1)**:**174-179.

3. Wong Y-W, Ashwood ER, Gordon W, Ash KO. ICP-MS quantitation of lead in blood collected on paper. *Clin Chem.* 1995;41:[Abstract No. 43].

4. Holtrop TG, Yee HY, Simpson PM, Kauffman RE. A community outreach lead screening program using capillary blood collected on filter paper [published erratum appears in *Arch Pediatr Adolesc Med*. 1998 Oct;152(10):991]. *Arch Pediatr Adolesc Med*. 1998;152(5)**:**455-458.

5. Moyer TP, Nixon DN, Ash KO. Filter paper lead testing. *Clin Chem.* 1999;45(12)**:**2055-2056.

6. Srivuthana K, Yee HY, Bhambhani K, Elton RM, Simpson PM, Kauffman RE. A new filter paper method to measure capillary blood lead level in children. *Arch Pediatr Adolesc Med.* 1996;150(5)**:**498-502.

7. Stanton NV, Maney JM, Jones R. Evaluation of filter paper blood lead methods: results of a pilot proficiency program. *Clin Chem.* 1999;45(12)**:**2229-2235.

8. Cernik AA. Determination of blood lead using a 4-0 mm paper punched disc carbon sampling cup technique. *Br J Ind Med*. 1974;31:239-244.

9. Weltz B. Symbols and units for integrated absorbance in electrothermal atomic absorption spectrometry (ET-AAS) *Spectrochim Acta Part B*. 1992;47(8):1043-1044.

Related NCCLS Publications*

C38-A **Control of Preanalytical Variation in Trace Element Determinations; Approved Guideline (1997).** This document provides guidelines for patient preparation, specimen collection, transport, and processing for the analysis of trace metals in a variety of biological matrices.

C42-A **Erythrocyte Protoporphyrin Testing; Approved Guideline (1996).** This document describes recommendations for the measurement, reporting, and interpretation of erythrocyte protoporphyrin using hematofluorometric and extraction measurement methods.

GP16-A **Routine Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline (1995).** This document provides descriptions of routine urinalysis test procedures that address materials and equipment, macroscopic examinations, clinical analyses, and microscopic evaluations. Additional information outlining specimen collection, acceptable specimen criteria, and storage considerations is also included.

H3-A4 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fourth Edition (1998).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children. Also included are recommendations on order of draw.

H4-A4 **Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture; Approved Standard—Fourth Edition (1999).** A consolidation of H4-A3 and H14-A2, this standard provides detailed descriptions and explanations of proper collection techniques, as well as hazards to patients from inappropriate specimen collection by skin puncture procedures.

LA4-A3 **Blood Collection on Filter Paper for Neonatal Screening Programs; Approved Standard—Third Edition (1997).** This document provides techniques for specimen collection; specifications for specimen matrix and shipment; and requirements for the specimen collection kit.

M29-A **Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline (1997).** This document provides guidance on the risk of transmission of hepatitis virus and human immunodeficiency viruses in the laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

NRSCL8-A **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document provides standard definitions for guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

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

Related NCCLS Publications (Continued)

NRSCL13-A **The Reference System for the Clinical Laboratory: Criteria for Development and Credentialing of Methods and Materials for Harmonization of Results; Approved Guideline (2000).** This document contains procedures for developing and evaluating definitive methods, reference methods, designated comparison methods, and reference materials to provide a harmonized clinical measurement system.

NOTES

NCCLS ▼ 940 West Valley Road ▼ Suite 1400 ▼ Wayne, PA 19087 ▼ USA ▼ PHONE 610.688.0100
FAX 610.688.0700 ▼ E-MAIL: exoffice@nccls.org ▼ WEBSITE: www.nccls.org ▼ ISBN 1-56238-437-6