CLINICAL AND LABORATORY STANDARDS INSTITUTE®

October 2013

C40-A2

Measurement Procedures for the Determination of Lead Concentrations in Blood and Urine; Approved Guideline—Second Edition

This document provides guidance for the measurement of lead concentrations in blood and urine, including specimen collection, measurement by graphite furnace atomic absorption spectrometry, anodic stripping voltammetry, and inductively coupled plasma mass spectrometry. It also includes guidelines for quality assurance and quality control, and information on proficiency testing programs and laboratory certification.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

Clinical and Laboratory Standards Institute

Setting the standard for quality in clinical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing clinical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement, but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advancements in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential, and may be submitted by anyone, at any time, on any document. All comments are addressed according to the consensus process by a committee of experts.

Appeals Process

If it is believed that an objection has not been adequately addressed, the process for appeals is documented in the CLSI Standards Development Policies and Process document.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For further information on committee participation or to submit comments, contact CLSI.

Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: 610.688.0100
F: 610.688.0700
www.clsi.org
standard@clsi.org

ISBN 1-56238-891-6 (Print)
ISBN 1-56238-892-4 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

C40-A2
Vol. 33 No. 13
Replaces C40-A
Vol. 21 No. 9

Measurement Procedures for the Determination of Lead Concentrations in Blood and Urine; Approved Guideline—Second Edition

Volume 33 Number 13

Uttam Garg, PhD, DABCC
Montserrat González Estecha, MD, PhD
Robert L. Jones, PhD
Jennifer Lowry, MD
Nelly Manay, PhD
Robb Morse
Heather Mowers
Patrick J. Parsons, PhD, C. Chem, FRSC
Erlo Roth, MD, MBA, FCAP
Noel V. Stanton, MS
Douglas F. Stickle, PhD, DABCC, FACB

Abstract

Clinical and Laboratory Standards Institute document C40-A2—*Measurement Procedures for the Determination of Lead Concentrations in Blood and Urine; Approved Guideline—Second Edition* is intended for use by members of 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; and lead determination by graphite furnace atomic absorption spectrometry, anodic stripping voltammetry, and inductively coupled plasma mass spectrometry. It also addresses reference materials, QC procedures, and laboratory policy.

Clinical and Laboratory Standards Institute (CLSI). *Measurement Procedures for the Determination of Lead Concentrations in Blood and Urine; Approved Guideline—Second Edition*. CLSI document C40-A2 (ISBN 1-56238-891-6 [Print]; ISBN 1-56238-892-4 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2013.

Suggested Citation

CLSI. *Measurement Procedures for the Determination of Lead Concentrations in Blood and Urine; Approved Guideline—Second Edition.* CLSI document C40-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.

Proposed Guideline
April 1998

Approved Guideline
June 2001

Approved Guideline—Second Edition
October 2013

ISBN 1-56238-891-6 (Print)
ISBN 1-56238-892-4 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

Committee Membership

Consensus Committee on Clinical Chemistry and Toxicology

David G. Grenache, PhD, DABCC, FACB
Chairholder
University of Utah, ARUP Laboratories
Salt Lake City, Utah, USA

Loralie J. Langman, PhD
Vice-Chairholder
Mayo Clinic
Rochester, Minnesota, USA

Julianne Cook Botelho, PhD
Centers for Disease Control and Prevention
Atlanta, Georgia, USA

Johanna Camara, PhD
National Institute of Standards and Technology
Gaithersburg, Maryland, USA

Yung W. Chan, MT(ASCP)
FDA Center for Devices and Radiological Health
Rockville, Maryland, USA

Corinne R. Fantz, PhD, DABCC
Emory University
Atlanta, Georgia, USA

T. Scott Isbell, PhD, DABCC, FACB
Nova Biomedical Corporation
Chicago, Illinois, USA

Joseph Passarelli
Roche Diagnostics GMBH
Indianapolis, Indiana, USA

Jessie Shih, PhD
Abbott
Abbott Park, Illinois, USA

Document Development Committee on Measurement Procedures for the Determination of Lead in Blood and Urine

Uttam Garg, PhD, DABCC
Chairholder
Children's Mercy Hospitals and Clinics
Kansas City, Missouri, USA

Montserrat González Estecha, MD, PhD
Hospital Clínico Universitario San Carlos
Madrid, Spain

Nelly Manay, PhD
University of the Republic of Uruguay
Montevideo, Uruguay

Heather Mowers
Ahlstrom Filtration LLC
Mt. Holly Springs, Pennsylvania, USA

Patrick J. Parsons, PhD
C. Chem, FRSC
New York State Department of Health
Albany, New York, USA

Douglas F. Stickle, PhD, DABCC, FACB
Jefferson University Hospitals
Philadelphia, Pennsylvania, USA

Staff

Clinical and Laboratory Standards Institute
Wayne, Pennsylvania, USA

Luann Ochs, MS
Senior Vice President – Operations

Ron S. Quicho
Staff Liaison

Patrice E. Polgar
Project Manager

Megan L. Tertel, MA
Editor

Acknowledgment

CLSI and the Consensus Committee on Clinical Chemistry and Toxicology gratefully acknowledge the following individuals for their help in preparing the second edition of this document:

Robert L. Jones, PhD
Centers for Disease Control and Prevention
Atlanta, Georgia, USA

Jennifer Lowry, MD
Children's Mercy Hospitals and Clinics
Kansas City, Missouri, USA

Robb Morse
Magellan Biosciences
Chelmsford, Massachusetts, USA

Erlo Roth, MD, MBA, FCAP
Hinsdale Pathology Associates
Hinsdale, Illinois, USA

Noel V. Stanton, MS
Wisconsin State Laboratory of Hygiene
Madison, Wisconsin, USA

Contents

Abstract i

Committee Membership iii

Foreword vii

1 Scope 1

2 Standard Precautions 1

3 Terminology 2

3.1 A Note on Terminology 2
3.2 Definitions 2
3.3 Abbreviations and Acronyms 5

4 Clinical Significance of Lead Concentration Measurements 7

4.1 Absorption of Lead and Its Internal Distribution Within the Body 8
4.2 Toxic Effects of Exposure to Lead in Children and Adults 8
4.3 Concentration of Lead in Blood Deemed Safe for Children/Adults 11
4.4 Use of Blood Lead Concentration Measurements as a Marker of Lead Exposure 12
4.5 Use of Urinary Lead Concentration Measurements in the Mobilization Test 13
4.6 *In Vivo* X-ray Fluorescence Measurements of Bone Lead 13
4.7 Use and Analysis of Erythrocyte Protoporphyrin 13
4.8 Reference Intervals for Lead in Blood and Urine 14

5 Measurement Procedures for Lead Concentration 15

5.1 Early Methods for the Determination of Lead Concentration 15
5.2 Flame Atomic Absorption Spectrometry and Methyl Isobutyl Ketone Extraction 15
5.3 Delves-Cup Flame Atomic Absorption Spectrometry Method 15
5.4 Anodic Stripping Voltammetry 16
5.5 Graphite Furnace Atomic Absorption Spectrometry 16
5.6 Mass Spectrometric Methods 17
5.7 Future Approaches to Blood Lead Concentration Measurements 20

6 Procedure for Collecting Blood and Urine for Lead Concentration Determination (see Section 10.3) 21

6.1 Capillary Blood Samples 21
6.2 Procedure for Collecting Venous Blood Samples 27
6.3 Procedure for Collecting Urine Samples 29

7 Procedure for Lead in Blood and Urine by Graphite Furnace Atomic Absorption Spectrometry 31

7.1 Principle of Measurement and Method Summary 31
7.2 Instrumentation Requirements 31
7.3 Reagents and Other Materials 34
7.4 Preparation of Reagents, Calibration Standards, and Samples for Analysis 35
7.5 Furnace Operation and Maintenance 38
7.6 Calculations 39
7.7 Troubleshooting 40

8 Procedures for Lead in Blood by Anodic Stripping Voltammetry 43

8.1 Method Principle 43
8.2 Instrumentation and Procedure 44

Contents (Continued)

9 Reference Materials and Quality Control for Lead in Blood and Urine 45

9.1 Reference Materials 45
9.2 Quality Control 51

10 Laboratory Policy....53

10.1 Laboratory Certification and Proficiency Testing 53
10.2 Follow-up and Rescreening 53
10.3 General Policies for Lead Analyses (see Section 5) 55

References....58

Appendix A. Laboratory Certification and Proficiency Testing in the United States....67

Appendix B. Procedure for Checking Materials and Specimen Collection Supplies for Lead Contamination....70

Appendix C. Electronic Reporting Policies for US Blood Lead Laboratories....78

The Quality Management System Approach 80

Related CLSI Reference Materials 81

Foreword

Blood lead (BPb) concentrations are used to assess lead exposure. In the United States in 1991, the Centers for Disease Control and Prevention (CDC) lowered BPb concentrations deemed harmful to children from 25 µg/dL (1.21 µmol/L) to 10 µg/dL (0.48 µmol/L).[1] The CDC estimates that 250 000 US children aged 1 to 5 years have BPb levels greater than 10 µg/dL (0.48 µmol/L). At this lower BPb concentration, the erythrocyte protoporphyrin test, once widely used as a screening test for lead exposure, became redundant due to its poor sensitivity for identifying low lead exposure. The CDC recommends direct measurement of BPb concentration to evaluate lead toxicity. It is now increasingly recognized that concentrations below 10 µg/dL (0.48 µmol/L) have a number of adverse health effects.

In 2012, the CDC Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) recommended, and the CDC concurred, that the term "blood lead level of concern" of 10 µg/dL (0.48 µmol/L), which was last revised in 1991, be eliminated and replaced with a new reference value for BPb in children 1 to 5 years of age. The ACCLPP recommendation was based on compelling scientific evidence that BPb concentrations less than 10 µg/dL (0.48 µmol/L) are associated with IQ deficits, attention-related behaviors, and poor academic achievement in young children.

With support from the CDC, the ACCLPP recommended that an elevated BPb level be defined as a reference value based on the 97.5th percentile of the BPb distribution among children 1 to 5 years old according to the US National Health and Nutrition Examination Survey (NHANES). The current NHANES 97.5th percentile BPb value is 5 µg/dL (0.24 µmol/L). It was also recommended that the reference value be updated every four years based on the most recent population-based BPb surveys among children. Based on the NHANES data, approximately 450 000 children in the United States have BPb levels higher than 5 µg/dL (0.24 µmol/L). Detailed information on these changes can be found on the CDC website.[2,3]

The CDC last updated its recommendations for screening young children for lead poisoning in November 1997.[4] The 1997 CDC document addressed specific concerns about the extent to which universal or targeted screening of children should be, or can be, implemented. As part of the release of the 1997 document, the CDC provided specific advice and materials to BPb laboratories that complement the guidelines proposed in this edition of C40. These materials may also be downloaded from the CDC's website.[5]

In 2012, the ACCLPP also recommended updating the CDC 1997 document regarding appropriate follow-up actions for confirmed, elevated BPb concentrations. The ACCLPP recommendations can be found on the CDC website.[2] The CDC is expected to operationalize the ACCLPP recommendations and publish revised guidance. Information on laboratory accreditation in the United States is also provided in Appendix A, along with details of proficiency testing (PT) (or external quality assessment) programs for BPb in Australia, Canada, the United States, and throughout Europe.

In 1991, established US PT requirements for BPb trueness were tightened to reflect the current improvements in methodology and the lower concentrations of BPb that were deemed harmful. Some laboratories using older methods for BPb were unable to maintain proficiency and were required to improve their method performance. Many were understandably concerned that the analytical technology for making accurate, contamination-free measurements of low concentrations of lead in capillary blood samples did not exist. Since the release of the 1991 CDC statement, it has been shown that current measurement procedures can easily measure BPb concentrations below 10 µg/dL (0.48 µmol/L) with acceptable trueness and precision. The performance of analytical methods at a reference value of 5 µg/dL (0.24 µmol/L) or lower requires evaluation. Measurement trueness continues to improve as evidenced by the performance of participating laboratories in numerous QA and PT programs.

This edition of C40 replaces the first edition of the approved guideline, C40-A, which was published in 2001. The most significant additions since the 2001 edition include:

- The clinical significance of lead concentrations < 10 µg/dL (0.48 µmol/L)
- The definition of elevated BPb in children based on a reference value of 5 µg/dL (0.24 µmol/L)
- General information on sample preparation and analysis by inductively coupled plasma mass spectrometry
- The use of filter paper in blood collection for lead screening

Key Words

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

Measurement Procedures for the Determination of Lead Concentrations in Blood and Urine; Approved Guideline—Second Edition

1 Scope

This document is intended for use by members of the clinical laboratory testing community involved in the collection and measurement of lead in blood and urine. A background section on the clinical significance of lead concentration measurements is included to help laboratorians and others understand the context in which these measurements are made. Sample collection and measurement by the three principal measurement procedures currently in routine use are included and listed below.

1. *Electrothermal atomic absorption spectrometry (ETAAS),* also widely known as *graphite furnace atomic absorption spectrometry (GFAAS).* Instrumentation for GFAAS is available from many commercial sources. Because instruments vary significantly among manufacturers, a generic measurement procedure is described in some detail.

2. *Anodic stripping voltammetry (ASV).* Commercial ASV instrumentation specifically for blood lead (BPb) concentration measurement is currently available from a single manufacturer. A detailed ASV procedure, which includes use of a proprietary reagent, is provided by the manufacturer. 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.

3. *Inductively coupled plasma mass spectrometry (ICP-MS).* ICP-MS is increasingly used for lead and trace element analysis. It is particularly useful for measuring low lead concentrations (< 10 µg/dL [0.48 µmol/L]), because the newer literature suggests that lead concentrations of < 10 µg/dL (0.48 µmol/L) have detrimental effects, and the reference level for BPb in children has been lowered to 5 µg/dL (0.24 µmol/L). ICP-MS is more sensitive than GFAAS and ASV. Because there are significant variations among makes and models of ICP-MS instrumentation, some general information and recommendations on sample preparation and analysis by ICP-MS are provided.

The document also includes guidelines for QA and QC, and information on proficiency testing (PT) programs and laboratory certification.

The analyst is free to choose which technique best suits the laboratory's needs, and may modify the recommended procedure to achieve accurate and precise results that meet scientific and regulatory requirements. 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 per the laboratory's needs and any applicable regulations.

2 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. The Centers for Disease Control and Prevention (CDC) addresses this topic in published guidelines that address the daily operations of diagnostic medicine in human and animal medicine while encouraging a culture of safety in the laboratory.[6] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and

