CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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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.

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

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

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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.

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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

for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[7]

3 Terminology

3.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI's consensus process for development and revision of standards and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

A hierarchy of terminology was agreed upon, involving ISO (www.iso.org), CEN (www.cen.eu), and CLSI (www.clsi.org).

Essentially, CLSI documents are obliged to harmonize with the most current edition of the *International Vocabulary of Metrology – Basic and General Concepts and Associated Terms* (VIM)[8] whenever an ambiguity in the interpretation or understanding of terms occurs. In the latest edition, many definitions have become more explicit and understandable, but the language of the VIM is difficult and compact. The VIM deals with general metrology and terminology that should be useful for most disciplines that measure quantities.

The understanding of a few terms has changed during the last decade as the concepts have developed. Particularly, *trueness* (measurement trueness) is defined as the closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value; and *precision* (measurement precision) is defined as closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. *Repeatability* and *reproducibility* are considered to be the extreme measures of precision, with repeatability being the smallest measure (same operator, method, equipment, time, and laboratory) and reproducibility being the largest (different operator, equipment, and laboratory).

The term *measurand* is used instead of *analyte* when referring to the quantity intended to be measured (component represented in the name of a measurable quantity) when its use relates to a biological fluid/matrix; the term *measurement procedure* replaces *analytical method* and *assay* for a set of operations, used in the performance of particular measurements according to a given method.

Verification focuses on whether specifications of a measurement procedure can be achieved, whereas *validation* verifies that the procedure is fit for purpose.

In addition, the term *primary reference (measurement) procedure* has replaced the term *definitive method* in C40.

3.2 Definitions

accuracy (measurement) – closeness of agreement between a measured quantity value and a true quantity value of a measurand (JCGM 200:2012)[8]; **NOTE 1:** The concept "measurement accuracy" is not

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The QSEs are as follows:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

C40-A2 addresses the QSE indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
		GP41 M29		GP41		X C24 C38 C42 EP05 EP17 GP16 GP41 GP42 NBS01					

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

C40-A2 addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
GP41 NBS01	X C38 GP16 GP41 GP42 NBS01	X GP16 GP41 NBS01	X GP16 GP41 NBS01	C42 GP16 GP41 NBS01	X C42 GP16 GP41	C42	X C42	NBS01

Related CLSI Reference Materials*

C24-A3 **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition (2006).** This document provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.

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 measurement of trace elements in a variety of biological matrices.

C42-A **Erythrocyte Protoporphyrin Testing; Approved Guideline (1996).** This document contains recommendations for the measurement, reporting, and interpretation of erythrocyte protoporphyrin using hematofluorometric and extraction measurement methods.

EP05-A2 **Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers' precision performance claims and determining when such comparisons are valid; as well as manufacturers' guidelines for establishing claims.

EP17-A2 **Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition (2012).** This document provides guidance for evaluation and documentation of the detection capability of clinical laboratory measurement procedures (ie, limits of blank, detection, and quantitation), for verification of manufacturers' detection capability claims, and for the proper use and interpretation of the different detection capability estimates.

GP16-A3 **Urinalysis; Approved Guideline—Third Edition (2009).** This document addresses procedures for testing urine, including materials and equipment; macroscopic/physical evaluation; chemical analysis; and microscopic analysis.

GP41-A6 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Sixth Edition (2007).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children.

GP42-A6 **Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition (2008).** This document provides a technique for the collection of diagnostic capillary blood specimens, including recommendations for collection sites and specimen handling and identification. Specifications for disposable devices used to collect, process, and transfer diagnostic capillary blood specimens are also included.

M29-A3 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

NBS01-A6 **Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard—Sixth Edition (2013).** This document highlights specimen collection methods, discusses acceptable techniques for applying blood drops or aliquots to the filter paper segment of the specimen collection device, and provides instructions on proper specimen handling and transport to ensure quality specimens are consistently obtained for newborn screening analysis.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

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