C42-A
Erythrocyte Protoporphyrin Testing; Approved Guideline

This document contains recommendations for the measurement, reporting, and interpretation of erythrocyte protoporphyrin using hematofluorometric and extraction measurement methods.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.
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 medical 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 managed according to the consensus process by a committee of experts.

**Appeals Process**

When it is believed that an objection has not been adequately considered and responded to, the process for appeals, documented in the CLSI Standards Development Policies and Processes, is followed.

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 additional 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: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org
Erythrocyte Protoporphyrin Testing; Approved Guideline

Abstract

_Erythrocyte Protoporphyrin Testing; Approved Guideline_ (CLSI document C42-A) is a comprehensive document for use by laboratorians who perform erythrocyte protoporphyrin (EP) determinations; its aim is to reduce/eliminate the lack of uniformity in current measurement practices. The biochemistry and pathology of EP are discussed, the history of EP determinations is summarized, and the applications of the test are defined. The document recommends the adoption of a specific molar absorptivity constant for the standardization of EP methods and the universal adoption of reporting units expressed as the molar ratio of protoporphyrin to heme. Detailed methods for the measurement of EP by extraction and hematofluorometry are included, and the interpretation of EP results is discussed.


The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If your organization is not a member and would like to become one, and to request a copy of the catalog, contact us at: Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: customerservice@clsi.org; Website: www.clsi.org.
Erythrocyte Protoporphyrin Testing; Approved Guideline

Volume 16 Number 8

Noel V. Stanton, M.S.
Dean Brown, M.S.
Peter R. Dallman, M.D.
Elaine Gunter, M.T.(ASCP)
Robert F. Labbe, Ph.D.
Patrick J. Parsons, Ph.D.
Ray Yip, M.D.
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>i</td>
</tr>
<tr>
<td>Committee Membership</td>
<td>v</td>
</tr>
<tr>
<td>Active Membership</td>
<td>vii</td>
</tr>
<tr>
<td>Foreword</td>
<td>xiii</td>
</tr>
<tr>
<td>1 Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2 Scope</td>
<td>1</td>
</tr>
<tr>
<td>3 Precautions</td>
<td>1</td>
</tr>
<tr>
<td>3.1 Universal Precautions</td>
<td>1</td>
</tr>
<tr>
<td>3.2 Instrument Hazards</td>
<td>1</td>
</tr>
<tr>
<td>4 Biochemistry</td>
<td>2</td>
</tr>
<tr>
<td>4.1 Definitions</td>
<td>2</td>
</tr>
<tr>
<td>4.2 Abbreviations</td>
<td>4</td>
</tr>
<tr>
<td>4.3 Biosynthesis</td>
<td>4</td>
</tr>
<tr>
<td>4.4 Regulation</td>
<td>4</td>
</tr>
<tr>
<td>5 History of Analytical Determination of Protoporphyrin</td>
<td>4</td>
</tr>
<tr>
<td>6 Applications of the Erythrocyte Protoporphyrin Test</td>
<td>6</td>
</tr>
<tr>
<td>6.1 Lead Poisoning</td>
<td>7</td>
</tr>
<tr>
<td>6.2 Iron Deficiency</td>
<td>8</td>
</tr>
<tr>
<td>7 Millimolar Absorptivity: A Historical Perspective</td>
<td>9</td>
</tr>
<tr>
<td>8 Recommended Procedure for the Quantitative Fluorometric Determination</td>
<td>10</td>
</tr>
<tr>
<td>8.1 Principle of the Method</td>
<td>10</td>
</tr>
<tr>
<td>8.2 Apparatus</td>
<td>11</td>
</tr>
<tr>
<td>8.3 Protoporphyrin IX Standard Solutions</td>
<td>12</td>
</tr>
<tr>
<td>8.4 Specimen Requirements</td>
<td>14</td>
</tr>
<tr>
<td>8.5 Method for the Determination of Erythrocyte Protoporphyrin by Extraction</td>
<td>14</td>
</tr>
<tr>
<td>8.6 Calculations</td>
<td>16</td>
</tr>
<tr>
<td>8.7 Reference Intervals</td>
<td>16</td>
</tr>
<tr>
<td>8.8 Quality Assurance</td>
<td>16</td>
</tr>
<tr>
<td>8.9 HPLC Analysis</td>
<td>18</td>
</tr>
<tr>
<td>9 Front-Surface Fluorometry (Hematofluorometry)</td>
<td>18</td>
</tr>
<tr>
<td>9.1 Principle of Operation</td>
<td>18</td>
</tr>
</tbody>
</table>
Foreword

In the past twenty years, there has been tremendous change in the application and analytic methodology of erythrocyte protoporphyrin (EP) testing. Before the early 1970s, testing for EP was generally restricted to research settings. The discovery of an association between EP and blood lead created a substantial demand for the test and, in 1978, the Centers for Disease Control (now the Centers for Disease Control and Prevention) recommended EP as the primary screening test for childhood lead poisoning. It was also learned that EP is a sensitive indicator of iron status, which provides another useful application for the EP test.

This interest in EP led to the development of assay methods suitable for the clinical laboratory: first a two-step extraction procedure followed by conventional fluorometry, and later the introduction of compact, dedicated front-face fluorometers (hematofluorometers), which allowed an immediate, simple, and inexpensive test result using a drop of whole blood.

However, these analytic methods are not without problems. When hematofluorometers (HF) were introduced, primary standards did not exist, and the available secondary standards proved to be unreliable. Generally, the standardization of HF is traceable to extraction results, but discrepancies between these methods are well documented in the literature. In fact, the standardization of extraction methods has been contentious, due, in large part, to the lack of stability of protoporphyrin in solution and a lack of consensus as to the molar absorptivity of the molecule. In addition, a lack of uniformity in reporting units exists, with results reported either as some ratio of protoporphyrin to hemoglobin or as a simple weight per whole blood volume concentration.

This document addresses these problems. It provides recommendations for uniform procedures, recommends the universal adoption of molar ratio reporting units, and gives detailed procedures for analysis by both extraction and hematofluorometry.

The concentration of blood lead considered acceptable has been reduced below the point at which EP is affected, which resulted in a decline in demand for this test. EP remains useful, however, in determining the duration and extent of lead exposure, as well as having utility in the characterization of iron status. This NCCLS document provides a thorough discussion of all aspects involved in the laboratory determination of this clinically important analyte.

Key Words

Erythrocyte protoporphyrin, hematofluorometer, iron deficiency, lead poisoning, zinc protoporphyrin.
Erythrocyte Protoporphyrin Testing; Approved Guideline

1 Introduction

Because there is an overall lack of uniformity in measurement practices for erythrocyte proto-porphyrin (EP) testing, the analysis of EP is an area that is in need of guidelines. Differences in the basis for measurement between the two most commonly used methods, extraction and hematofluorometry, have also been a source of confusion. This document provides recommended methods for achieving valid results using either method. To improve comparability between methods, enhance diagnostic utility, and conform to Système International d’Unités (SI units), the subcommittee recommends that all EP measurements be reported in units that compare the abundance of EP to the abundance of heme in the specimen (micromole per mole).

As of 1993, most laboratories were standardizing EP measurements based on an inaccurate reference value. The committee recommends that the dimethyl ester (DME) hydrolysis preparation technique be used for the preparation of protoporphyrin IX (PPIX) standards and that $297 \, \text{L} \cdot \text{mmol}^{-1} \cdot \text{cm}^{-1}$ be adopted as the correct millimolar absorptivity ($m_c$) for PPIX. Detailed directions are provided. The revised value for $m_c$ will result in new reference intervals, which are given.

2 Scope

This document is useful to all laboratories that perform EP tests; users of hematofluorometry in nonlaboratory settings will benefit from its use as well.

Recommended methods for extraction (Section 8) and hematofluorometry (Section 9) of EP test procedures are given. The millimolar absorptivity ($m_c$) of protoporphyrin IX (PPIX) is redefined (Section 7). This will result in changes in calibration and reference intervals for EP. New reference intervals are given (Section 10). The history of method development for EP (Section 5) and the biochemistry of porphyrins (Section 4) are described. Terminology (Section 4), sections on the causes of elevated porphyrin results (Section 6) and interpretation (Section 10), and a brief description of liquid chromatography for the differentiation of the various porphyrins (Section 8.9) are provided. The appendices address the preparation of QA materials and the availability of proficiency-testing programs.

Although NCCLS documents generally use units that are fully acceptable within the Système International d’Unités (SI), these do not always coincide with the units recommended by the International Union of Pure and Applied Chemistry (IUPAC) and by the International Federation of Clinical Chemistry (IFCC) for reporting results of clinical laboratory measurements. NCCLS documents also include the IUPAC/IFCC-recommended units of volume (L) and substance (molecular) concentration (mol/L) in parentheses, where appropriate.

3 Precautions

3.1 Universal Precautions

Because it is often impossible to know which might be infectious, all patient blood specimens are to be treated with universal precautions. Guidelines for specimen handling are available from the U. S. Centers for Disease Control and Prevention [MMWR 987;36(suppl 2S):2S–18S]. NCCLS document M29-T2, Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue—Second Edition; Tentative Guideline, deals specifically with this issue.

3.2 Instrument Hazards

As described in the manufacturer’s instrument manual, and in relevant sections of this document, when using instrumentation, take precautions to avoid safety hazards and biohazards. For additional information, refer to NCCLS document I17-P, Protection of Laboratory Workers from Instrument Biohazards; Approved Guideline.
4. Biochemistry

While reading the following sections, refer to Figure 1 for relevant molecular structures and to Figure 2 for an outline of the heme biosynthetic pathway.1

4.1 Definitions

Erythrocyte protoporphyrin, n- The total non-heme (iron-free) protoporphyrin (3, 7, 12, 17-tetra-methyl-8, 13-divinyl-2, 18-porphinedipropionic acid) present in erythrocytes. It includes both metal-free protoporphyrin and zinc protoporphyrin (ZP). Erythrocyte protoporphyrin is a product of blood porphyrin analysis in which the commonly used acid extraction solvent liberates the chelated zinc to yield the larger pool of metal-free protoporphyrin. This term is recommended as a replacement for “free erythrocyte protoporphyrin (FEP),” which should be reserved for specific reference to native, metal-free porphyrin.

Ferrochelatase, n- The enzyme that catalyzes the chelation of iron (III) by protoporphyrin with the liberation of two protons. Alternatively, the enzyme utilizes zinc ion as the metal substrate in states of insufficient iron. Ferrochelatase can be inhibited transiently in severe, acute lead poisoning. It is not inhibited significantly in chronic lead poisoning (EC 4.99.1.1).

Hematofluorometry, n- An analytical procedure based on the measurement of front surface fluorescence of blood (erythrocytes) with exposure to long wavelength ultraviolet
Related NCCLS Publications


I17-P  Protection of Laboratory Workers from Instrument Biohazards; Proposed Guideline (1991). I17-P offers guidelines for the protection of those who use and maintain laboratory equipment from infectious diseases transmitted in human body fluids and tissue that might contaminate equipment.

M29-T2  Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue—Second Edition; Tentative Guideline (1991). M29-T2 provides guidance on the risk of transmission of hepatitis B virus and the human immunodeficiency virus in the laboratory. Specific precautions for preventing the transmission of bloodborne infection during clinical anatomical laboratory procedures are addressed.

**Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.**
Explore the Latest Offerings From CLSI!

As we continue to set the global standard for quality in laboratory testing, we are adding products and programs to bring even more value to our members and customers.

By becoming a CLSI member, your laboratory will join 1,600+ other influential organizations all working together to further CLSI’s efforts to improve health care outcomes. You can play an active role in raising global laboratory testing standards—in your laboratory, and around the world.

Find out which membership option is best for you at www.clsi.org/membership.

Find what your laboratory needs to succeed! CLSI U provides convenient, cost-effective continuing education and training resources to help you advance your professional development. We have a variety of easy-to-use, online educational resources that make eLearning stress-free and convenient for you and your staff.

See our current educational offerings at www.clsi.org/education.

When laboratory testing quality is critical, standards are needed and there is no time to waste. eCLIPSE™ Ultimate Access, our cloud-based online portal of the complete library of CLSI standards, makes it easy to quickly find the CLSI resources you need.

Learn more and purchase eCLIPSE at clsi.org/eCLIPSE.

For more information, visit www.clsi.org today.