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November 1996

C42-A

Erythrocyte Protoporphyrin Testing; Approved Guideline

SAMPLE

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.

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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.

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Contents

Page

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

9.2 Materials and Reagents 19

9.3 Specimen Requirements 19

9.4 Instrument Calibration 20

9.5 Operating Procedure: General 20

9.6 Operating Procedure: Instruments That Use a Derivatizing Reagent 21

9.7 Results 22

9.8 Interferences 23

9.9 Quality Assurance/Quality Control (QA/QC) 23

10 Interpretation of Erythrocyte Protoporphyrin Values 26

10.1 Erythrocyte Protoporphyrin Reporting Units 26

10.2 Reference Intervals 26

10.3 Interpretation of Erythrocyte Protoporphyrin Results 27

Appendix A: Preparation of Quality Control Materials for Extraction Methods 29

Appendix B: Proficiency Testing and Laboratory Certification 30

References 32

Bibliography 37

Summary of Comments and Subcommittee Responses 38

Related NCCLS Publications 41

SAMPLE

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 ($m\epsilon$) for PPIX. Detailed directions are provided. The revised value for $m\epsilon$ 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 ($m\epsilon$) 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*.

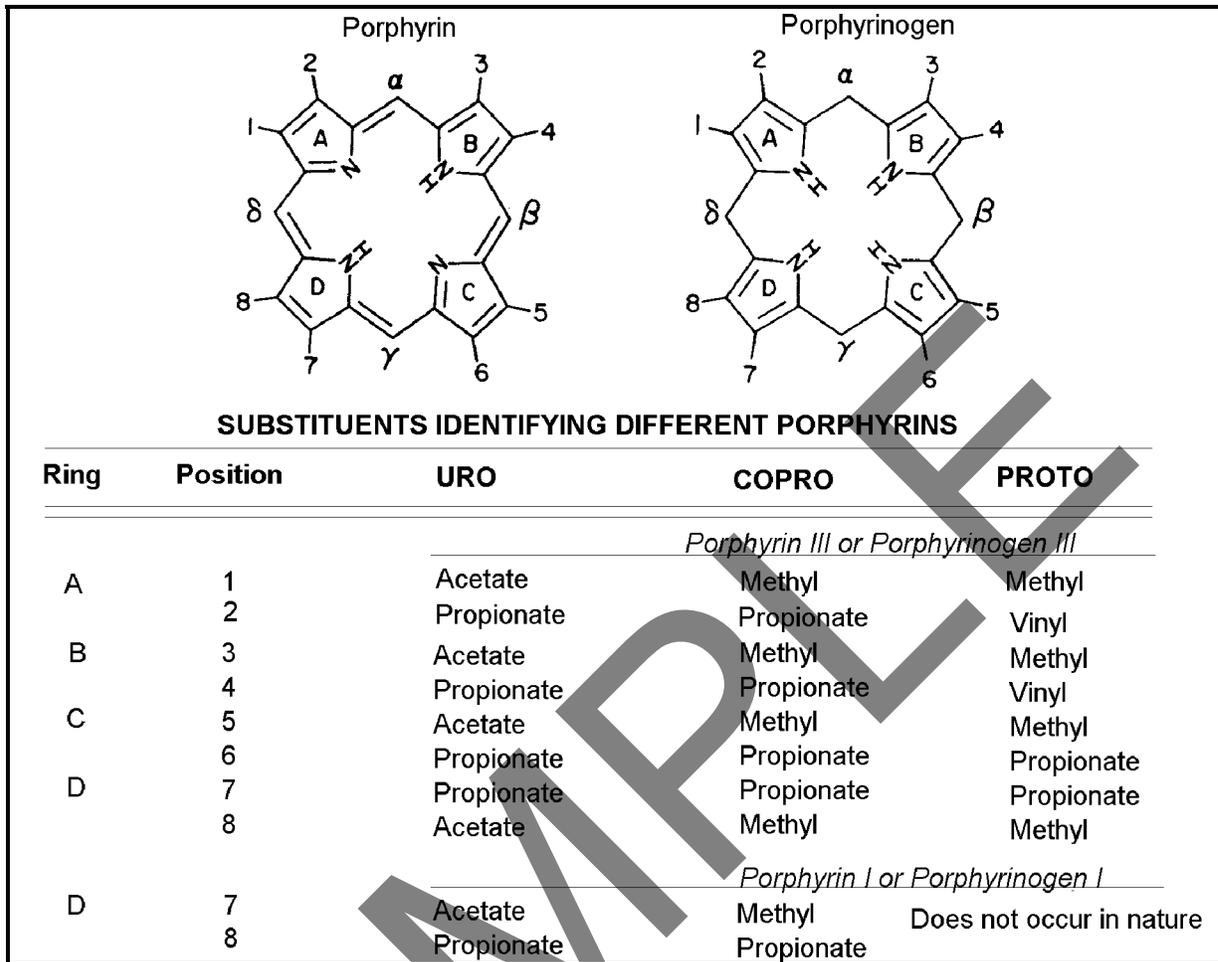


Figure 1. Structure of porphyrin and porphyrinogen and a list of the substituents that identify the different porphyrins. From Labbe RF, Lamon JM. Chemistry of porphyrins. In: Tietz NW, ed. *Textbook of Clinical Chemistry*. Philadelphia: WB Saunders, 1986. Reprinted with permission.

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-porphine-dipropionic 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 (II) 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**

- C28-A** **How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline (1995).** C28-A gives a protocol for the determination of reference intervals for defined populations to help with interpretation of laboratory data.
- 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.

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