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Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood; Approved Standard—Third Edition

This document describes the principle, materials, and procedure for reference and standardized hemoglobin determinations. It includes specifications for secondary hemiglobincyanide (HiCN) standards.

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

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Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood; Approved Standard—Third Edition

Abstract

NCCLS document H15-A3, *Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood; Approved Standard—Third Edition*, describes the measurement of blood hemoglobin using the hemiglobincyanide (HiCN) method, including composition of, and criteria for, the reagent and the calibration of photometers. The procedures described in H15 are required for whole blood calibration procedures for automated hematology analyzers; are necessary in the evaluation of instruments and alternative methods for the determination of hemoglobin concentration; and should be applied when patient red blood cell measurements are used for calibration and control of hematology analyzers. A separate section contains specifications for, and spectral characteristics of, HiCN solutions suitable for use as standards. The document enables users to achieve accurate hemoglobin concentration values for diagnostic or reference purposes. Producers of HiCN calibration standards can use the document as a guideline; users will have the information necessary to check for the content and purity of those materials.

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Brian S. Bull, M.D.
Berend Houwen, M.D., Ph.D.
John A. Koepke, M.D.
Elkin Simson, M.B., Ch.B., M.Med.
Onno W. van Assendelft, M.D., Ph.D.

NCCLS

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Committee Membership

Area Committee on Hematology

Eugene L. Gottfried, M.D. **Chairholder**	**San Francisco General Hospital** **San Francisco, California**
Charles F. Arkin, M.D. **Vice-Chairholder**	**Boston University Medical Center** **Boston, Massachusetts**
William R. Swaim, M.D.	Burnsville, Minnesota
Luc van Hove, M.D., Ph.D.	Abbott Laboratories Santa Clara, California
Onno W. van Assendelft, M.D., Ph.D.	Centers for Disease Control and Prevention Atlanta, Georgia
Joan D. Wiseman, M.T.(ASCP), CT	Becton Dickinson VACUTAINER Systems Franklin Lakes, New Jersey

Advisors

J. David Bessman, M.D.	University of Texas Medical Branch Galveston, Texas
Brian S. Bull, M.D.	Loma Linda University Medical Center Loma Linda, California
Bruce H. Davis, M.D.	Maine Medical Center Research Institute South Portland, Maine
Virgil F. Fairbanks, M.D.	Mayo Clinic Rochester, Minnesota
Berend Houwen, M.D., Ph.D.	Loma Linda University Medical Center Loma Linda, California
J. Heinrich Joist, M.D., Ph.D.	St. Louis Coagulation Consultants St. Louis, Missouri
John A. Koepke, M.D.	Durham, North Carolina
Jack Levin, M.D.	VA Medical Center San Francisco, California
Albert Rabinovitch, M.D., Ph.D.	Kinnelon, New Jersey
T. Vincent Shankey, M.D., Ph.D.	Loyola University Medical Center Maywood, Illinois

Advisors (Continued)

Elkin Simson, M.B., Ch.B., M.Med.	Mount Sinai Medical Center New York, New York
Diane I. Szamosi, M.A., M.T.(ASCP), SH	Becton Dickinson VACUTAINER Systems Franklin Lakes, New Jersey
Jennifer K. McGeary, M.T.(ASCP), M.S.H.A. *Staff Liaison*	NCCLS Wayne, Pennsylvania
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Contents

Contents (Continued)

Foreword

"Although the determination of the amount of haemoglobin is one of the most important of all chemical tests of the blood, as a rule it is one which is determined with less care and by methods more inaccurate than those in use for any other constituent of the body."[1] There have been many advances in methodology and in instrumentation since the above statement was published. The biggest step forward is undoubtedly the development and acceptance of a reference method for hemoglobin determination and the availability of calibration standards.

In 1958, a Panel on the Establishment of a Hemoglobin Standard, Division of Medical Sciences—National Research Council, reviewed several photometric methods used for determining hemoglobin levels and concluded that, for several reasons, the best method was that in which hemoglobin was measured after conversion to cyanmethemoglobin:

> *The method involves dilution with a single reagent. All forms of hemoglobin likely to occur in circulatory blood, with the exception of sulfhemoglobin, are determined. The color is suitable for measurement in filter as well as in narrow-band spectrophotometers, because its absorption band at a wavelength of 540 nanometers is broad and relatively flat. Standards prepared from either crystalline hemoglobin or washed erythrocytes and stored in a brown glass container and in sterile condition are stable for at least nine months (change 2%).*

Criteria for a United States cyanmethemoglobin standard were then published.[2]

Work continued, primarily in Europe, on the determination of the relative molecular mass of hemoglobin and on the accurate determination of the (quarter) millimolar absorptivity of hemiglobincyanide (cyanmethemoglobin). In 1963, a Standardizing Committee of the European Society of Haematology was founded; in 1964, this committee became the International Committee for Standardization in Haematology (ICSH) and an ICSH Expert Panel on Haemoglobinometry was formed to draw up recommendations. Recommendations were accepted at the International Congress of Haematology in 1966 and published in 1967. Meanwhile, the National Institute of Public Health in the Netherlands prepared and made available, on behalf of ICSH, an international hemiglobincyanide (HiCN) reference solution, one lot of which was accepted by the World Health Organization (WHO) as the International HiCN Reference Preparation (WHO Techn. Rep. Series 384: 85, 1968). WHO subsequently accepted further batches of HiCN reference solution as the second, third, etc., International HiCN Reference Preparation (now, reference standard). The international HiCN reference solutions are controlled by laboratories in Italy, Japan, the Netherlands, the United Kingdom, and the United States.

Other methods for the determination of hemoglobin were described over the past decade:[3-5] The HiCN method, however, remains the benchmark against which all other methods are evaluated.

In the United States the Health Care Financing Administration, U.S. Public Health Service, Department of Health and Human Services, in February 1992, published the final rule of the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88).[6] In this final ruling, clinical laboratory tests are categorized as either "waived tests," "tests of moderate complexity," or "tests of high complexity." Waived tests include the "screening" of blood for hemoglobin concentration below or above a certain cut-off level by means of copper sulfate testing. On the recommendation of the Clinical Laboratory Improvement Advisory Committee, determination of hemoglobin concentration using whole blood collected into disposable cuvettes that contain reagents in dried form and measuring with a simple, portable, dedicated photometer, was recently added to the list of waived tests. Tests of moderate complexity include the determination of hemoglobin as part of automated hematology procedures, with or without white cell differential counting, *that do not require operator intervention during the analytic process* and do not require an analyst to

Foreword (Continued)

interpret a histogram or scattergram. All other methods to determine hemoglobin concentration, including the (manual) reference procedure, are considered to be tests of high complexity.

The first part of this standard contains guidelines for the accurate measurement of blood hemoglobin concentration using the hemiglobincyanide method. It includes composition of, and criteria for, the reagent and the calibration of photometers; routine filtration of HiCN solutions to fully reduce all background turbidity; and a need to demonstrate, in the reference procedure, that a particular instrument-cuvette combination does not show apparent light absorption by the reagent. The procedures described in H15 are required for whole blood calibration procedures for automated hematology analyzers; are necessary in the evaluation of instruments and alternative methods for the determination of hemoglobin concentration; and should be applied when patient red blood cell measurements are used for calibration and control of hematology analyzers. This section is intended to provide all laboratory personnel with a thorough understanding of the HiCN method and to enable them to obtain accurate hemoglobin concentration values for diagnostic and for reference purposes.

The second part of this standard contains specifications for, and the spectral characteristics of, HiCN solutions suitable for use as secondary photometer calibration material. It includes the calculation of the HiCN content from spectrophotometric measurements. This section was written as a guideline for producers of HiCN calibration materials and to allow users of such materials to check for content and purity of the HiCN solutions.

Please note the following changes in this document: in Section 6.2 on Reagents, a description of interferences in the method has been included (Section 6.2.6). In Part II, a section on storage of HiCN standards (Section 3.7) and a section on source material other than human blood to prepare HiCN standards (Section 5) have been included.

Key Words

Calibrator, hemiglobincyanide (HiCN), hemoglobin, hemoglobinometry, reference method

Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood; Approved Standard—Third Edition

Part I. Reference and Selected Procedures for the Determination of Hemoglobin Concentration

1 Introduction

Recommendations for the determination of hemoglobin concentration in human blood were prepared by the International Council (previously Committee) for Standardization in Haematology (ICSH) in 1978,[7] 1987,[8] and 1996.[9] The photometric determination of hemiglobincyanide [HiCN; cyanmethemoglobin (see Section 5)] is recommended as the reference method:

> *If any other method is used in routine measurement (for example, photometric determination of oxyhaemoglobin or haemiglobinazide; iron determination), it should be adjusted to obtain comparability with the haemiglobincyanide method. The determination of haemoglobin as haeminchloride (acid haematin) is not recommended because of the unreliability of this method.*[7,8]

2 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 for 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*.

3 Scope

Part I of this standard describes the determination of hemoglobin concentration in human blood by the HiCN (cyanmethemoglobin) method. Accurate determination of hemoglobin concentration is:

- required for whole blood calibration procedures of automated hematology analyzers;
- necessary for the assignment of values to control materials used in hemoglobin measurement procedures;
- necessary in the evaluation of instruments and alternative methods for the measurement of hemoglobin concentration;