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H07-A3

Procedure for Determining Packed Cell Volume by the Microhematocrit Method; Approved Standard—Third Edition

This document describes a standard microhematocrit method for determining packed cell volume; specifications for recommended materials and information on potential sources of error are also included.

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

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Procedure for Determining Packed Cell Volume by the Microhematocrit Method; Approved Standard—Third Edition

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Abstract

Clinical and Laboratory Standards Institute document H07-A3—*Procedure for Determining Packed Cell Volume by the Microhematocrit Method* describes a standard method for direct measurement of packed cell volume (PCV). The standard is intended for reference use by clinical laboratory personnel and by manufacturers of instruments that determine PCV. The method can also be used (with appropriate precautions as described in the document) in the clinical laboratory for diagnostic purposes, for monitoring a patient's response to therapy, and for evaluating instruments and other methods for determining PCV; the standard should be used for whole blood calibration procedures of hematology analyzers.

The document gives detailed specifications of the materials to be used in the procedure, contains information for calibrating the centrifuge and reading device, and includes information on verification of calibration. Expression of results, generally accepted reference values, and potential sources of error are given.

Clinical and Laboratory Standards Institute (CLSI). *Procedure for Determining Packed Cell Volume by the Microhematocrit Method; Approved Standard—Third Edition*. CLSI document H07-A3 (ISBN 1-56238-413-9). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2000.

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Contents

Foreword

Methods used to determine the relative volume of the red cellular constituents of blood include indicator dilution techniques, measuring the relative electrical impedance of cells and their supporting medium, and centrifugation.

Determination of the relative red cell volume by the hemoglobin ratio technique[1] is reliable and gives absolute values. The technique is not affected by the incorporation of white blood cells into the red cell volume, by plasma trapping and/or by red cell dehydration effects,[2] but is too time-consuming for routine use. Its major contribution to the routine determination of the relative red cell volume stems from its ability to aid in the selection of appropriate dimensions and materials for the manufacture of glass microcapillary tubes.

Indicator dilution techniques[3] have not proved useful as reference methods and differences in the amount of trapped plasma, depending upon the indicator used, have been described.[4]

Measuring electrical impedance of red cells gives a relative value that may be influenced by shape and orientation of the cells in plasma or diluting medium, by resistivity changes of plasma in disease, by other blood constituents, and by variability of instrument calibration.

Measuring light scatter of red cells gives a relative value that may be influenced by light absorption of the cells because of hemoglobin concentration, by other blood constituents, and by variability of instrument calibration. Measuring light scatter at two different angles will decrease the influence of cell hemoglobin content on the measurement.

Methods based on centrifugation include macrohematocrit[5] (first described in 1929 and no longer in use) and microhematocrit.[6] Standard microhematocrit methods require about 50 µL of blood for each determination[6,7,8]; certain special micromethods[9] require even less blood.

The standardized procedure for the microhematocrit method discussed in this document was chosen by the subcommittee because of its widespread availability, acceptable level of precision, and the relatively simple apparatus used. Identified errors caused by plasma trapping and red cell dehydration that are known to approximately compensate each other are also described. The subcommittee believes that the method is the most acceptable, readily available method for use as a benchmark for evaluation purposes and, especially with dipotassium ethylenediaminetetraacetic acid as anticoagulant (see Section 6.1), for assigning values to whole blood calibration material.

The term “hematocrit” originally referred to the apparatus or the procedure used to determine the volume fraction of the erythrocytes in whole blood. The terms “packed cell volume” and “hematocrit” are often, however, considered synonymous. The subcommittee has chosen the term "packed cell volume" (PCV), to describe the quantity measured by centrifugation and has reserved the term “hematocrit” to describe materials and/or methods used in the determination.

Both the tentative- and the earlier approved-level editions of H07 have been widely reviewed by the clinical laboratory testing community and have generated numerous comments. The subcommittee thanks all commentors for their recommendations. (See especially the “**NOTE**” regarding the joint FDA/NIOSH/CDC Safety Advisory in Section 5.2 of the document.) Each comment has been carefully reviewed and changes have been made where appropriate; however, not all viewpoints could be accommodated. Comments and subcommittee responses are included in this document.

Key Words

Hematocrit, hematocrit by hemoglobin ratio, microhematocrit, packed cell volume (PCV), plasma trapping, relative volume of red cells

Procedure for Determining Packed Cell Volume by the Microhematocrit Method; Approved Standard—Third Edition

1 Introduction

The packed cell volume (PCV) is the measure of the ratio of the volume occupied by the red cells to the volume of whole blood in a sample of capillary, venous, or arterial blood. The ratio is measured after appropriate centrifugation[6,10] and is expressed as a decimal fraction.

The PCV is an easily obtained measure for detecting anemia or polycythemia and can be useful in estimating changes in hemodilution or hemoconcentration. The PCV is used, together with the red cell count, in calculating the mean cell volume (MCV) and, together with the hemoglobin content, in calculating the mean corpuscular hemoglobin concentration (MCHC).

Direct measurement of PCV may be done by centrifugation.[6,7,8] Indirect measurements of the PCV are made by some (semi) automated instruments; methods include determination of red cell volume and red cell count by electrical conductivity measurements, or by optical extinction measurements, on a cell-by-cell basis. The PCV is then derived from these two measurements. These methods, not considered correct in the strictest meaning of the word, are generally accepted substitutes as part of the "automated complete blood count;" the measured quantity is commonly referred to as the "hematocrit."

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 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

This document describes the determination of the packed (red) cell volume by centrifugation.

Determination of the PCV by centrifugation is:

- required for whole blood calibration of instrumental methods;
- applicable in evaluating instruments and alternative methods for determining PCV;
- applicable in the routine hematology laboratory (with appropriate precautions as described in the document) for diagnostic purposes and for monitoring progress of therapy, especially when the nature of the sample, e.g., presence of cold agglutinins, may cause inaccuracies in the (automated) method in routine use.

NOTE: Commercially available microhematocrit systems that use capillary tubes not made of glass, smaller volumes of blood, different centrifugation times, or different centrifugation speeds may produce results that are fully acceptable for diagnostic or screening purposes, or for therapy monitoring. (See Sections 5.2, 6.2, and 6.4 for additional information.) Manufacturers' instructions must be followed for specific systems.

Thus, this standard is useful for all clinical laboratory personnel and for manufacturers of instruments that determine PCV.

4 Definitions[a]

Buffy coat, *n* - A yellowish-white layer of leukocytes and platelets that forms on top of the column of red blood cells upon centrifugation of whole blood. **NOTES:** a) Defined as "a blood component rich in leukocytes and platelets, suspended in a small volume of plasma from the same donation"; b) It is obtained either by separation from whole blood or by cytopheresis.

Hematocrit, HCT, *n* – See **packed cell volume**.

Mean cell volume, MCV, *n* - The average volume of the red blood cells in a given blood sample; expressed in femtoliter, 10^{-15} L. **NOTE:** MCV was previously reported in cubic micrometer, μm^3. The MCV is calculated as follows:

$$\text{MCV(fl)} = \frac{\text{PCV(as fraction)}}{\text{Number red cells per L}}$$

Mean corpuscular hemoglobin concentration, MCHC, *n* - The average hemoglobin concentration within the red blood cells. **NOTE:** The MCHC, expressed as the amount of hemoglobin (in grams) per deciliter of red cells (or per liter of red cells)[11,12] is calculated as follows:

$$\text{MCHC (g/dL)} = \frac{\text{Hemoglobin Concentraton (g/dL)}}{\text{PCV (as fraction)}}$$

Microhematocrit method, *n* - The determination of the packed cell volume (PCV) using a small quantity of whole blood, a capillary tube, and a high-speed centrifuge.

Packed cell volume, **PCV**, *n* - The measure of the ratio of the volume occupied by the red blood cells to the volume of whole blood, expressed as a fraction. **NOTE**: The term "hematocrit" has been, and is often, used for this quantity.

Parallax, *n* - The apparent displacement of an observed object due to a change in the position of the observer, or by the observer's use of both eyes versus one eye or the other for the observation.

Relative centrifugal field, RCF, *n* - The outward-directed centrifugal acceleration of an object moving in a circle at constant angular velocity; **NOTES:** a) The term "rotative centrifugal force" is widely used for RCF. However the actual quantity—relative centrifugal field—is dimensionless; thus, it is not a force and should not be called a force; b) This can be confused with revolutions per minute (rpm). The numerical measure of the RCF is calculated as follows:

$$\text{RCF}\,(g_n) = 0.00001118\,\text{r} \cdot \text{N}^2$$

[a] Some of these definitions are found in NCCLS Document NRSCL8—*Terminology and Definitions for Use in NCCLS Documents.* For complete definitions and detailed source information, please refer to the most current edition of that document.

Related NCCLS Publications*

H1-A4 **Evacuated Tubes and Additives for Blood Specimen Collection; Approved Standard—Fourth Edition (1996)**. *American National Standard.* This standard discusses requirements for blood collection tubes and additives including heparin, EDTA, and sodium citrate.

H3-A4 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved StandardXFourth Edition (1998).** This standard discusses procedures for collecting diagnostic blood specimens by venipuncture including line draws, blood culture collection, and venipuncture in children. It also includes recommendations on order of draw.

H4-A4 **Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture; Approved StandardXFourth Edition (1999)**. This document provides a technique for the collection of diagnostic blood specimens by skin puncture, including recommendations for collection sites and specimen handling and identification. Specifications for disposable devices used to collect, process, and transfer diagnostic blood specimens obtained by skin puncture are also included.

H15-A2 **Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood—Second Edition; Approved Standard (1994).** *American National Standard.* This standard describes the principle, materials, and procedure for reference and standardized hemoglobin determinations. Included are specifications for secondary hemiglobincyanide (HiCN) standards.

H18-A2 **Procedures for the Handling and Processing of Blood Specimens; Approved Guideline (1999).** This document includes criteria for preparing an optimal serum or plasma sample and for the devices used to process blood specimens.

H26-A **Performance Goals for the Internal Quality Control of Multichannel Hematology Analyzers; Approved Standard (1996).** This standard discusses recommended performance goals for analytical accuracy and precision based on mathematical models for the following measurements: hemoglobin concentration, erythrocyte count, leukocyte count, platelet count, and mean corpuscular volume.

M29-A **Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline (1997).** This document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

NRSCL8-A **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document provides standard definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

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

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