



H20-A2

Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition

SAMPLE

This document is a reference method for the evaluation of automated differential counters, based on the visual differential count.

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

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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

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Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition

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John A. Koepke, MD
Onno W. Van Assendelft, PhD
Larry J. Brindza, MPA
Bruce H. Davis, MD
Bernard J. Fernandes, MD
Amy S. Gewirtz, MD
Albert Rabinovitch, MD, PhD

Abstract

Clinical and Laboratory Standards Institute document H20-A2—*Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition* evaluates automated and semiautomated hematology instruments for their capability to perform an acceptable leukocyte (WBC) differential count. The standard focuses on WBC found in blood films. The standard presents a detailed description of an acceptable manual-visual WBC differential count, which serves as the reference for the instrumental differential counter. The types of abnormalities for inclusion are outlined.

A statistical method is also outlined, allowing for the determination of the performance of the test method in qualitative, as well as quantitative abnormalities.

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SAMPLE

Foreword

This document was first developed in 1981 as a proposed standard (H20-P), when automated and semi-automated instruments were initially created to perform leukocyte (WBC) differential classification. Early devices were glass slide-based, but then flow methods became dominant. This standard presents a detailed description of an acceptable manual-visual WBC differential count, which will serve as the reference method for the instrument systems.

WBC differential counts (either visual¹ or instrumental²) should have medically acceptable false-negative rates for unusual or abnormal conditions. In addition, however, they would be expected to have economically feasible false-positive rates.

The method as outlined is laborious and time-consuming. In its complete form, it may not be acceptable to many end-user laboratories. It is expected that manufacturers of WBC differential systems will use this standard to establish performance specifications. There are simplified versions of this standard, requiring relatively few specimens and no complicated statistical procedures.³ Use of alternate methods should be confirmed by the laboratory director, and other approaches may be acceptable in establishing how the instrument system performs compared to the traditional manual microscopic method.

Statistical studies are somewhat confounded by the commonly used method of reporting differentials (i.e., the proportional or percentage system). Absolute concentrations of circulating WBC are the preferable method of reporting, since those are the medically important values, rather than percentages.

Another area for considerable discussion, within the committee and in the entire field of laboratory medicine, is defining the “differential blood count.” Definitions vary from an enumeration of the major WBC subgroups (granulocytes, lymphocytes, and monocytes) to a very comprehensive review of all of the so-called formed elements, including erythrocytes and platelets. This document is limited to WBC normally found in the blood, including subdifferentiation of lymphocytes and neutrophils, plus the requirement that an “other” category be included for all other nucleated cells found in the blood.

Much of the information included in this document can be useful to the routine hematology laboratory, either in the production of accurate white cell differential counts or for incorporation into quality control procedures. As an example, the production of good blood films and their evaluation have been detailed in this document.

Advances in instrumentation have resulted in the possibility of extending the use of these devices to *quantitatively* measure the nucleated cells in the blood, which are currently flagged. For example, nucleated red blood cells can now be accurately counted on several analyzers, and these have been approved for use in clinical laboratories without the necessity of confirmation by blood film review.

This document replaces the approved standard, H20-A, which was published in 1992. Several changes have been made in this edition; chief among them is the introduction of a new orthogonal regression method for comparing duplicate data, in Section 12. The document has also been updated to include the following: a new examiner qualification process in Section 6.6.5, revision of Section 9.2, Data Acquisition to clarify the instructions for randomization of the morphology examination slides, Table 4 has been updated with the new NHANES data, and the method of arbitration for resolving discrepant findings of the morphologists has been updated.

Key Words

Basophil, blood film (differential leukocyte count), differential counting, eosinophil, leukocyte, lymphocyte, lymphocyte (variant form), monocyte, neutrophil (band form), neutrophil (segmented)

Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition

1 Scope

These recommendations cover performance testing of leukocyte (WBC) differential counting. Only those WBC found in normal (nondiseased) individuals will be addressed. These cell types are: neutrophils (segmented), neutrophils (band forms), lymphocytes (normal), lymphocytes (variant forms), monocytes, eosinophils, and basophils. If not identified, the system should appropriately flag other cells as abnormal, suspect or unclassified, or as nucleated red blood cells (NRBC).

Some devices may group several cell types into a single category. For example, segmented and band neutrophils, eosinophils, and basophils may be combined as granulocytes.

2 Introduction

2.1 Automated WBC Differential Counters

Automated WBC differential counters relieve the clinical laboratory of a labor-intensive activity.⁴ Because predetermined criteria are substituted for visual perception of laboratory personnel with varying skill and training, automation should improve reproducibility of the results. An opportunity also exists to improve the precision of the results by performing counts on many more cells than can be conveniently classified by human visual examination. A detailed list of hematology analyzers, published in December 2005, is available as a source of information.⁵

2.2 Classification of Automated Devices for WBC Differential Counts

2.2.1 Differential Counting Techniques

There are several different techniques for differential counting, including computerized image processing, flow methods, and other methods. The WBC types identified by these techniques are comparable, although not always identical.

2.2.2 Automated Devices

Several different levels of automated devices have been developed. Examples and intended uses of these different automated devices include the following:

- (1) automated cell locators and classifiers that tabulate the usually circulating cells and flag for review any abnormal WBC or other variations from normal;
- (2) classifiers of normal and abnormal WBC, which are suitable for screening purposes;
- (3) classifiers of normal and abnormal WBC, which are suitable for diagnostic purposes; and
- (4) devices for qualitatively and/or quantitatively determining patterns (size, shape, and/or staining) of formed elements in human blood (WBC, erythrocytes [RBC], and/or platelets [PLT]) in addition to one of the preceding uses.

2.3 Limitations

This publication is limited to cells that normally circulate in the human bloodstream plus a category for other cells that does not necessarily require identifying these cells. Thus, flagging clinically abnormal samples for visual review is an integral part of performance evaluation. Opinions differ regarding acceptable WBC reference ranges, complicating this identification. Disagreement also exists on the desirability of proportional (percentage) as compared to absolute counts. To address these considerations fairly, methods in this standard must be used with reference range criteria determined by the user.

2.4 Causes of Variability in WBC Counts

2.4.1 Sources of Variation

Sources of variation for a laboratory measurement may be divided into preanalytical, analytical, biological-physiological, and pathological. One or a combination of these variables may interfere.

2.4.2 Routine vs. Optimal Testing Times

Information gathered during routine testing may differ from data compiled under optimal conditions and supervision. Both sets of data are valid samplings of truth; however, performance data gathered during optimal testing times are not necessarily reproduced during routine testing. These methods of evaluation more closely resemble optimal than routine conditions.

3 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 infectious agents and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (Garner JS. Hospital Infection Control Practices Advisory Committee. Guideline for isolation precautions in hospitals. *Infect Control Hosp Epidemiol.* 1996;17:53-80). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of CLSI document M29—*Protection of Laboratory Workers From Occupationally Acquired Infections*.

4 Definitions

In the context of this publication, the terms listed below are defined as follows:

abnormality – **1)** an abnormal condition, state, or quality; irregularity; deviation; **2)** *In Hematology*, a clinically significant alteration in the distribution of mature cell types, or the presence of immature or abnormal cell types at or above clinically significant levels or fractions.

accuracy (clinical or diagnostic) – the qualitative or quantitative expression of a particular WBC differential finding in terms of sensitivity, specificity, efficiency, positive predictive value, and negative predictive value; **NOTE:** It is the separate predictive value statements that are medically meaningful.

accuracy (of measurement) – closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93).⁶

The Quality 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 approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any healthcare service’s path of workflow (i.e., 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 quality system essentials (QSEs) are:

- | | | | |
|--|--|---|--|
| Documents & Records
Organization
Personnel | Equipment
Purchasing & Inventory
Process Control | Information Management
Occurrence Management
Assessments—External
and Internal | Process Improvement
Customer Service
Facilities & Safety |
|--|--|---|--|

H20-A2 addresses the quality system essentials (QSEs) indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
				H3	X EP5 EP10 GP10 H1 H3 H4 H18 H26 M29	H1		EP10	H26		H3 M29

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*.

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

H20-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/NCCLS Publications 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
H3	H1 H3 H4	H3 H18	X H3 H18	X H18	X			

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*.

Related CLSI/NCCLS Publications*

- EP5-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 for comparing the resulting precision estimates with manufacturer’s precision performance claims and determining when such comparisons are valid; as well as manufacturer’s guidelines for establishing claims.
- EP10-A3** **Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition (2006).** This guideline addresses experimental design and data analysis for preliminary evaluation of the performance of a measurement procedure or device.
- GP10-A** **Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Plots; Approved Guideline (1995).** This document provides a protocol for evaluating the accuracy of a test to discriminate between two subclasses of subjects where there is some clinically relevant reason to separate them. In addition to the use of ROC plots, the importance of defining the question, selecting the sample group, and determining the “true” clinical state are emphasized.
- H1-A5** **Tubes and Additives for Venous Blood Specimen Collection; Approved Standard—Fifth Edition (2003).** This document contains requirements for venous blood collection tubes and additives, including technical descriptions of ethylenediaminetetraacetic acid (EDTA), sodium citrate, and heparin compounds used in blood collection devices.
- H3-A5** **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fifth Edition (2003).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children. It also includes recommendations on order of draw.
- H4-A5** **Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Fifth Edition (2004).** 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.
- H18-A3** **Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Third Edition (2004).** 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 document addresses performance goals for analytical accuracy and precision for multichannel hematology analyzers; the relationship of these goals to quality control systems and medical decisions; and recommendations for minimum calibrator performance and the detection of measurement errors.
- 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.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

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950 West Valley Road, Suite 2500, Wayne, PA 19087 USA

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P: 610.688.0100 Toll Free (US): 877.447.1888 F: 610.688.0700

E: customerservice@clsi.org www.clsi.org