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H20-A2
Vol. 27 No. 4
Replaces H20-A
Vol. 12 No. 1

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

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

Advancing Quality in Healthcare Testing

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H20-A2
ISBN 1-56238-628-X
Volume 27 Number 4 ISSN 0273-3099

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

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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.

Clinical and Laboratory Standards Institute (CLSI). *Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition.* CLSI document H20-A2 (ISBN 1-56238-628-X). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.

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Suggested Citation

(Clinical and Laboratory Standards Institute. *Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition.* CLSI document H20-A2 [ISBN 1-56238-628-X]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.)

Proposed Standard
July 1981

Tentative Standard
August 1984

Approved Standard
March 1992

Approved Standard—Second Edition
January 2007

ISBN 1-56238-628-X
ISSN 0273-3099

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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 visual[1] or instrumental[2]) 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)

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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.

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