CLINICAL AND LABORATORY STANDARDS INSTITUTE®

3rd Edition

CLSI H20™

Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrumental Methods

CLSI H20 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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Replaces CLSI H20-A2

Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrumental Methods

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Abstract

CLSI H20—*Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrumental Methods* describes a reference method for evaluating automated and semiautomated hematology instruments for their capability to perform an acceptable leukocyte (ie, WBC) differential count. CLSI H20 focuses on WBCs found in blood smears. CLSI H20 presents a detailed description of an acceptable manual (visual) WBC differential count, which serves as the reference method for the instrumental differential counter. The types of abnormalities for inclusion are outlined. A sample statistical method is also outlined, enabling the determination of the test method's performance for qualitative, as well as quantitative, abnormalities.

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Foreword

CLSI H20 was first developed in 1981, when automated and semiautomated instruments were initially created to perform leukocyte (white blood cell) differential classification. Early devices were glass slide–based, but then flow methods became dominant. CLSI H20 presents a detailed description of an acceptable manual (visual) leukocyte differential count, which serves as the reference method for the instrument systems. An alternative reference method using multiparametric flow cytometric technique is also described.

Leukocyte differential counts (either visual[1] or instrumental[2]) should have medically acceptable false-negative rates for unusual or abnormal conditions. Additionally, they are expected to have economically feasible false-positive rates.

The method outlined is laborious and time-consuming. In its complete form, it might not be acceptable to many end-user laboratories. It is expected that manufacturers of leukocyte differential systems will use CLSI H20 to establish performance specifications. There are simplified versions of CLSI H20, requiring relatively few specimens and no complicated statistical procedures.[3] Use of alternate methods should be confirmed by the laboratory director, and other approaches might be acceptable in establishing the instrument system's performance compared with the traditional manual microscopic method.

Statistical studies are somewhat confounded by the commonly used method of reporting differentials (ie, the proportional or percentage system). Absolute concentrations of circulating leukocytes are the preferable method of reporting, because those are the medically important values, although their calculation requires percentage quantification.

Another area of considerable discussion is defining the "differential blood count." Definitions vary from an enumeration of the major leukocyte subgroups (granulocytes, lymphocytes, and monocytes) to a comprehensive review of all the so-called formed elements, including erythrocytes and platelets. CLSI H20 is limited to leukocytes normally found in the blood of healthy individuals, 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 CLSI H20 can be useful to the routine hematology laboratory, either for production of accurate leukocyte differential counts or for incorporation into quality control procedures. For example, the production of good blood smears and their evaluation are detailed in CLSI H20.

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

Overview of Changes

CLSI H20-Ed3 replaces CLSI H20-A2, published in 2007. Several changes were made in this edition, including:

- Aligning terminology with international standards
- Providing updated consensus guidelines on qualifying new examiners to perform leukocyte differentials and on evaluating postexamination activities and clinical accuracy studies
- Expanding an alternative reference method approach (using multiparametric flow cytometric immunophenotyping procedures) to incorporate current and new practices and to reflect harmonization of the process
  - This approach uses recommended techniques for method calibration and validation, metrological traceability, and guidelines for cell identification of leukocytes normally found in the blood of healthy individuals.

NOTE: The content of CLSI H20 is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

basophil

blood film (peripheral blood smear)

differential counting (differential leukocyte count)

eosinophil

leukocyte

lymphocyte (normal)

lymphocyte (reactive form)

monocyte

neutrophil (band form)

neutrophil (segmented)

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Chapter 1

Introduction

Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrumental Methods

1 Introduction

1.1 Scope

CLSI H20 is intended for global use in medical laboratories to improve global harmonization of blood cell differential counts. This information might also be of interest to product manufacturers and regulatory agencies to aid in new instrument and assay development and the required validation and verification processes.
The recommendations in CLSI H20 cover performance testing of leukocyte (WBC) differential counting. Only leukocytes found in healthy (nondiseased) individuals are covered. These cell types are neutrophils (segmented), neutrophils (band forms), lymphocytes (normal), lymphocytes (reactive forms), monocytes, eosinophils, and basophils. If a cell type is not identified, the system should appropriately flag it as abnormal, suspect, or unclassified or as nucleated red blood cells (NRBCs).

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

Reference interval criteria for flagging abnormal samples are not included in CLSI H20. The user should determine these criteria. Newer reference methods by immunophenotypic definition of healthy blood cells are intended for method calibration and validation studies. These newer reference methods are not intended for routine clinical practice.

1.2 Background

1.2.1 Automated Leukocyte Differential Counters

Automated leukocyte differential counters relieve the medical laboratory of labor-intensive activity. Automation can improve the reproducibility of the results, because predetermined criteria are substituted for the visual perception of laboratory personnel, who have varied levels of skill and training. 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.

1.2.2 Classification of Automated Devices for Leukocyte Differential Counts

1.2.2.1 Differential Counting Techniques

There are 2 main approaches to differential leukocyte counting: manual microscopic techniques and automated methods. The leukocyte types identified by these techniques are comparable, although not always identical.

1.2.2.2 Automated Devices

There are several different techniques for automated differential leukocyte counting, including impedance, optical, flow cytometric, and computerized image processing. Numerous classes of automated devices have been developed that use these automated techniques to varying degrees. Examples and intended uses of these different automated approaches include:

- Automated cell locators and classifiers that tabulate typical circulating cells and flag for review any unusual or abnormal leukocytes

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