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Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition

This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.

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

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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Clinical and Laboratory Standards Institute

Advancing Quality in Health Care Testing

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- the authorization of a project
- the development and open review of documents
- the revision of documents in response to comments by users
- the acceptance of a document as a consensus standard or guideline.

Most documents are subject to two levels of consensus—"proposed" and "approved." Depending on the need for field evaluation or data collection, documents may also be made available for review at an intermediate consensus level.

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Approved An approved standard or guideline has achieved consensus within the health care community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (ie, that comments on earlier versions have been satisfactorily addressed), and to identify the need for additional consensus documents.

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Health care professionals in all specialties are urged to volunteer for participation in CLSI projects. Please contact us at customerservice@clsi.org or +610.688.0100 for additional information on committee participation.

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Abstract

Clinical and Laboratory Standards Institute document C28-A3c—*Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition* is written for users of diagnostic laboratory tests. It offers a protocol for determining reference intervals that meet the minimum requirements for reliability and usefulness. The guideline focuses on health-associated reference values as they relate to quantitative clinical laboratory tests. Included are various requirements for studies to determine reference values for a new analyte or a new analytical method of a previously measured analyte. Also discussed is the transfer of established reference values from one laboratory to another.

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Foreword

A measured or observed laboratory test result from a person (usually a patient) is compared with a reference interval for the purpose of making a medical diagnosis, therapeutic management decision, or other physiological assessment. The interpretation of clinical laboratory data is, therefore, a comparative decision-making process. For this decision-making process to occur, reference values are needed for all tests in the clinical laboratory, and the provision of reliable reference intervals is an important task for clinical laboratories and diagnostic test manufacturers. The reference values most commonly used (known as “normal values” and sometimes “expected values”) have traditionally been poorly defined and certainly not determined by a uniform process. It is now apparent that it is important to develop reference intervals using a more systematic process that takes into account the various influences on the measured laboratory test results.

A theory of reference values that provides definitions, principles, and procedures for the determination and use of reference values was developed by the Expert Panel on Theory of Reference Values (EPTRV) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the Standing Committee on Reference Values of the International Council for Standardization in Haematology (ICSH). The fruits of the tireless labors of these committees appear in a series of articles[1-6] that provide a rational approach and sound basis for the determination of reference values. These definitions also provided a basis for the development of this guideline. CLSI is indebted to the members of the IFCC committee and to the many other investigators who contributed to this discipline and upon whose knowledge it has drawn.

This guideline begins with definitions proposed by the EPTRV of the IFCC that are important to the discussion of reference values. An outline of the broad procedural protocol for establishing reference intervals is included, followed by specifics of each of the composite processes. Issues related to the reference subject selection process, the importance of preanalytical and analytical considerations, the calculation methods and requirements for estimating valid reference intervals, and the transference of reference intervals are discussed. Examples of the recommended estimation and calculation processes are provided. Finally, issues related to the presentation and use of reference intervals are discussed, followed by a brief section that examines a number of important but collateral reference value topics not amenable to inclusion in this document.

Key Words

Critical value, observed value, reference distribution, reference individual, reference interval, reference limit, reference population, reference sample group, reference value

Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition

1 Scope

This document provides diagnostic laboratories and diagnostic test manufacturers with updated guidelines for determining reference intervals for quantitative laboratory tests. It includes specific recommendations regarding procedures that can be used to establish and verify reliable reference intervals for use in clinical laboratory medicine. By following these recommendations, laboratories can provide reference intervals that are adequate and useful for clinical interpretation.

Issues related to the reference subject selection process, the importance of preanalytical and analytical considerations, the calculation methods and requirements for estimating valid reference intervals, and the transference of reference intervals are discussed. Examples of the recommended estimation and calculation processes are provided. Finally, issues related to the presentation and use of reference intervals are discussed, followed by a brief section that examines a number of important but collateral reference value topics not amenable to inclusion in this document.

2 Introduction

Since the last update to this document (2000), two notable trends have emerged in clinical laboratory practice to which the working group would like to call attention.

First, for some analytes, reference intervals have been replaced by ***decision limits***, established by national (or international) consensus. As examples, consider cholesterol and glycated hemoglobin. For such analytes, there is no need to establish *de novo*, or even to verify, the reference intervals. Rather, laboratories must concern themselves with the accuracy of the results they report; that is, that cholesterol values they report are not appreciably different from the values that are reported by a certified reference laboratory on the same samples. For such analytes, the onus falls on manufacturers to ensure their methods are traceable (see CLSI document X05[7]) and on individual laboratories to ensure they run those methods correctly (using peer group quality control [QC], proficiency testing, etc.).

Second, the working group recognizes the reality that, in practice, very few laboratories perform their own reference interval studies. As indicated in this document, the working group endorses its previous recommendation that the ***best*** means to ***establish*** a reference interval is to collect samples from a sufficient number of qualified reference individuals to yield a minimum of 120 samples for analysis, by nonparametric means, for each partition (eg, sex, age range).

The fact of the matter, though, is that few laboratories, or even manufacturers, do such studies. Often, if any study is done, far fewer individuals are used, with assumptions made about the underlying distributions and about the comparability among partitions. Sometimes (eg, electrolytes), instead of performing a new reference interval study, laboratories and manufacturers refer to studies done many decades ago, when both the methods and the population were very different.

For these reasons, the working group believes strongly that individual laboratories should focus more on ***verifying*** reference intervals established elsewhere, a much less formidable task. As noted in this document, this can be done in at least two practical ways:

(1) If a laboratory has previously established a reference interval for its own population, then it can verify that reference interval by ***transference***, using a CLSI/NCCLS document EP09[8] protocol (see Section 10). ***A major advantage of this option is there is no need to collect samples from***