C28-P3
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Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Proposed Guideline—Third Edition

PLEASE

This proposed document is published for wide and thorough review in the new, accelerated Clinical and Laboratory Standards Institute (CLSI) consensus-review process. The document will undergo concurrent consensus review, Board review, and delegate voting (ie, candidate for advancement) for 60 days.

Please send your comments on scope, approach, and technical and editorial content to CLSI.

Comment period ends

26 May 2008

The subcommittee responsible for this document will assess all comments received by the end of the comment period. Based on this assessment, a new version of the document will be issued. Readers are encouraged to send their comments to Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; Fax: +610.688.0700, or to the following e-mail address: customerservice@clsi.org.

COMMENT

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®
(Formerly NCCLS)

ifcc International Federation of Clinical Chemistry and Laboratory Medicine

Clinical and Laboratory Standards Institute

Advancing Quality in Health Care Testing

Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) is an international, interdisciplinary, nonprofit, standards-developing, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the health care community. It is recognized worldwide for the application of its unique consensus process in the development of standards and guidelines for patient testing and related health care issues. Our process is based on the principle that consensus is an effective and cost-effective way to improve patient testing and health care services.

In addition to developing and promoting the use of voluntary consensus standards and guidelines, we provide an open and unbiased forum to address critical issues affecting the quality of patient testing and health care.

PUBLICATIONS

A document is published as a standard, guideline, or committee report.

Standard A document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A standard may, in addition, contain discretionary elements, which are clearly identified.

Guideline A document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

Report A document that has not been subjected to consensus review and is released by the Board of Directors.

CONSENSUS PROCESS

The CLSI voluntary consensus process is a protocol establishing formal criteria for:

- 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.

Proposed A consensus document undergoes the first stage of review by the health care community as a proposed standard or guideline. The document should receive a wide and thorough technical review, including an overall review of its scope, approach, and utility, and a line-by-line review of its technical and editorial content.

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.

Our standards and guidelines represent a consensus opinion on good practices and reflect the substantial agreement by materially affected, competent, and interested parties obtained by following CLSI's established consensus procedures. Provisions in CLSI standards and guidelines may be more or less stringent than applicable regulations. Consequently, conformance to this voluntary consensus document does not relieve the user of responsibility for compliance with applicable regulations.

COMMENTS

The comments of users are essential to the consensus process. Anyone may submit a comment, and all comments are addressed, according to the consensus process, by the committee that wrote the document. All comments, including those that result in a change to the document when published at the next consensus level and those that do not result in a change, are responded to by the committee in an appendix to the document. Readers are strongly encouraged to comment in any form and at any time on any document. Address comments to Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

VOLUNTEER PARTICIPATION

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-P3—*Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Proposed 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.

Invitation for Participation in the Consensus Process

An important aspect of the development of this and all CLSI documents should be emphasized, and that is the consensus process. Within the context and operation of CLSI, the term "consensus" means more than agreement. In the context of document development, "consensus" is a process by which CLSI, its members, and interested parties (1) have the opportunity to review and to comment on any CLSI publication; and (2) are assured that their comments will be given serious, competent consideration. Any CLSI document will evolve as will technology affecting laboratory or health care procedures, methods, and protocols; and therefore, is expected to undergo cycles of evaluation and modification.

The Area Committee on Clinical Chemistry and Toxicology has attempted to engage the broadest possible worldwide representation in committee deliberations. Consequently, it is reasonable to expect that issues remain unresolved at the time of publication at the proposed level. The review and comment process is the mechanism for resolving such issues.

The CLSI voluntary consensus process is dependent upon the expertise of worldwide reviewers whose comments add value to the effort. At the end of a 60-day comment period, each subcommittee is obligated to review all comments and to respond in writing to all which are substantive. Where appropriate, modifications will be made to the document, and all comments along with the subcommittee's responses will be included as an appendix to the document when it is published at the next consensus level.

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; Proposed Guideline—Third Edition

1 Scope and Introduction

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 will meet the minimum, mandatory requirements for adequate reliability and usefulness.

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 X5[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 feels 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 an EP9[8] protocol (see Section 10). ***A major advantage of this option is that there is no need to collect samples from reference individuals.*** One can use existing patient samples, even from subjects not known to be healthy, thus overcoming one of the major obstacles in reference interval studies.

2) As an alternative, a laboratory can verify a reference range, established by more stringent techniques elsewhere, **by collecting as few as 20 samples from qualified, reference individuals.** As noted in Section 10, with the data from these samples in hand, one can do a simple binomial test, or one can apply more sophisticated tests to achieve better sensitivity and specificity. Whichever method one chooses, though, the important point is that, with as few as 20 samples from reference individuals, a laboratory can verify reasonably well the applicability of a reference interval to its own population and methodology.