CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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EP28-A3c

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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For further information on committee participation or to submit comments, contact CLSI.

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

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Abstract

Clinical and Laboratory Standards Institute document EP28-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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IFCC International Federation of Clinical Chemistry and Laboratory Medicine

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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***

***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 interval, established by more stringent techniques elsewhere, ***by collecting as few as 20 samples from qualified reference individuals.*** As noted in Section 11, 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, 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.

The CLSI working group is encouraged by other developments that should make the establishment of reference intervals less formidable.

- The working group urges all manufacturers to ensure their methods exhibit traceability to appropriate standards (see CLSI document X05[7] and ISO 17511[9]) when they exist. As a result, values for many assays from different laboratories should be interchangeable, which may make it possible to combine data from multiple sites to establish reference intervals, thereby reducing the burden on each laboratory to collect samples from as many as 120 individuals (see Section 6.2).

- The working group calls attention to computer-intensive procedures that permit increased precision and less stringent sample size requirements to establish reference intervals. If a laboratory has adequate statistical and computing competence, the working group encourages consideration of procedures that do not require 120 individuals to estimate reference limits and confidence intervals (CI) (see Section 9).

In summary, the working group believes ***every laboratory is more than capable of verifying the applicability of reference intervals to its own population*.** In addition, the working group strongly endorses the recommendations of the previous working group on the proper way to establish a reference interval, and it extends those recommendations by introducing recommendations about multicenter studies and modern statistical methods.

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 feature 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 US Centers for Disease Control and Prevention.[10] 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 CLSI document M29.[11]

4 Terminology

4.1 A Note on Terminology

The document begins with the definition of certain terms that are important to the discussion of reference values. The terminology adopted is proposed by the Expert Panel on Theory of Reference Values (EPTRV) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), which was carefully developed for a more systematic and unambiguous discussion. An outline of the broad

The Quality Management 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 document HS01—*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 health care service's path of workflow (ie, 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 QSEs are:

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

EP28-A3c addresses the QSEs indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessments—External & Internal	Process Improvement	Customer Service	Facilities & Safety
H11		H11	H11	H03	X C24 C49 EP09 GP16 H03 H04 H11 H18 H21 M29 X05				H11		H03 H11 M29

Adapted from CLSI document HS01—*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 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.

EP28-A3c 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 Reference Materials 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
H03 H11	GP16 H03 H04 H11 H21	GP16 H03 H11 H18 H21	GP16 H03 H11 H18	H03 H18	H03			

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

Related CLSI Reference Materials*

C24-A3 **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition (2006).** This guideline provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.

C49-A **Analysis of Body Fluids in Clinical Chemistry; Approved Guideline (2007).** This document provides guidance for the application of widely available measurement procedures for testing body fluids and for reporting and interpreting those results. It emphasizes defining the common clinical situations for this use; acceptable practice for measuring analytes without extended method verification for abnormal body fluid; influence of biologic and analytic variation on interpretation of results; and variability in comparing results between different instrument manufacturers. This document does not consider serum, plasma, whole blood, or fluids for which assays typically have performance claims in the measurement procedure documentation.

EP09-A2 **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (2002).** This document addresses procedures for determining the bias between two clinical methods, and the design of a method comparison experiment using split patient samples and data analysis.

GP16-A2 **Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline—Second Edition (2001).** This document addresses procedures for testing urine, including materials and equipment; macroscopic/physical evaluation; chemical analysis; and microscopic analysis. In addition, a step-by-step outline for collecting, transporting, and storing specimens is included.

H03-A6 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Sixth Edition (2007).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children.

H04-A6 **Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition (2008).** 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.

H11-A4 **Procedures for the Collection of Arterial Blood Specimens; Approved Standard—Fourth Edition (2004).** This document provides principles for collecting, handling, and transporting arterial blood specimens to assist with reducing collection hazards and ensuring the integrity of the arterial specimen.

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.

H21-A5 **Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition (2008).** This document provides procedures for collecting, transporting, and storing blood; processing blood specimens; storage of plasma for coagulation testing; and general recommendations for performing the tests.

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.

X05-R **Metrological Traceability and Its Implementation; A Report (2006).** This document provides guidance to manufacturers for establishing and reporting metrological traceability.

* 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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