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How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second 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 NCCLS consensus process.

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How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second Edition

Abstract

How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second Edition (NCCLS document C28-A2) 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 (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. We are indebted to the members of the IFCC committee and to the many other investigators who contributed to this discipline and upon whose knowledge we have 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.

Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to “standard precautions.” Standard precautions are new guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996; Vol 17;1:53-80.), [MMWR 1987;36(suppl 2S):2S-18S] and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure, refer to NCCLS document M29—*Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*.

Key Words

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

How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second Edition

1 Introduction and Scope

This document provides diagnostic laboratories, diagnostic test manufacturers, and users of clinical laboratory tests with guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests. It includes the methodological approaches and recommended procedures for establishing reliable reference intervals for use in clinical laboratory medicine. The recommendations contained in this document comprise a protocol for determining reference intervals that meet the minimum, mandatory requirements for adequate reliability and usefulness. There are situations that will require more guidance than these recommendations can provide. Such situations cannot be covered entirely in this brief document. However, in certain areas, the additional steps or efforts that would improve the reliability and accuracy of the reference interval determination are indicated. Because of the lack of uniformity in the data collection and in the methodology currently used for establishing reference intervals by clinical laboratory scientists and manufacturers, it is the subcommittee's hope that this document will provide a basic and uniform protocol for achieving a comparable level of reliability and a foundation for more elaborate studies.

The procedures for determining "health-associated" reference values or intervals derived from a reference sample group of persons who are in good health are the primary focus of the document. However, other types of reference values, for example, for other physiological or pathological conditions, could also be established in a similar manner. With attention to the selection of appropriate reference individuals and due consideration of preanalytical factors, the procedures outlined here can be followed for the determination of any type of reference interval. However, this document does not specifically address the process required to establish critical values or other medical decision limits, such as diagnostic cut-offs. These determinations require a different approach, in part, and are often based on the diagnostic sensitivity and specificity for a specific medical condition.

The various needs and requirements of reference value studies for different situations are also addressed, including:

- measurement of a new analyte
- measurement by a new or different analytical method of a previously known and measured analyte for which physiological data and other reference values may be available
- measurement of the same analyte by the same or comparable analytical method for which reference value studies from another laboratory or the manufacturer are available (transference).

The latter issue, which is referred to as “transference of reference values,” is complex. The validation of transference and the subsequent transfer of reference values will increasingly be an issue encountered by the clinical laboratory testing community as diagnostic test manufacturers and other laboratories provide appropriately determined reference value data. Approaches to this problem are not yet rigorous; consequently, this issue is discussed in terms of general recommendations and three acceptable approaches.

If a diagnostic laboratory, large or small, or a diagnostic test manufacturer has to establish a reference interval through a reference value study, the specific guidelines and procedures provided in this document should be followed. This document contains the minimum standards for an adequate and appropriate reference interval determination. If the facility is small and lacks the resources to conduct such an