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Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

This document provides guidance for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of the limits.

A guideline for global application developed through the NCCLS consensus process.

NCCLS...

Global Consensus Standardization for Health Technologies

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Abstract

NCCLS document EP17-A—*Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline* provides protocols for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of these limits. This document also provides guidance for determining lower limits of quantitation based on a laboratory's goals for performance at low-levels. This applies to all quantitative procedures, even if the reported result is qualitative. EP17-A is intended for use by clinical laboratories and by manufacturers of *in vitro* diagnostic tests.

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NCCLS

IFCC International Federation of Clinical Chemistry and Laboratory Medicine

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Foreword

Laboratory methods have many performance characteristics that must be understood and assessed for their appropriate use. The performance characteristics for any method describe the method's capability to reliably measure the amount of an analyte in a subject's sample (although for some analytes, reliable detection is sufficient). Two such critical performance characteristics are defined at the lower end of the measurement scale. The first is the smallest amount that the method can reliably detect to determine presence or absence of an analyte. This is the limit of detection (LoD). The second characteristic is the smallest amount the method can reliably measure quantitatively. This is the limit of quantitation (LoQ).

The limits of detection and quantitation are critical because detecting extremely small amounts of an analyte can be necessary to define disease states, screen for disease, identify significant exposure, or to reveal the presence or absence of toxins, pollutants, carcinogens, contaminants, infectious agents, and illicit drugs. Some of these applications may effectively use the examination method as a qualitative or semiquantitative (ordinal scale) procedure. Although the discrimination point, or cutoff value, for a qualitative method is rarely designated by the developer to be equivalent to the lowest detectable amounts of the analyte, knowledge of the limit of detection informs the choice of a cutoff, so the procedures in this document should be applicable.

The LoD and LoQ are also important in laboratory examinations for tumor markers, hormones, agents of infectious diseases, therapeutic drugs, and other tests where low values separate subjects into different disease or exposure categories. Because of the statistical sophistication of these protocols, it is expected that this document will be used primarily by manufacturers and clinical laboratory supervisors.

A Note on Terminology

NCCLS, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. NCCLS recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in NCCLS, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. In light of this, NCCLS recognizes that harmonization of terms facilitates the global application of standards and deserves immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

The Subcommittee on Limits of Detection has made every effort to use globally accepted terms wherever possible. These are described in Sections 2.3.1 and 2.3.2 which discuss some commonly used terms that are not employed in this document, and in Section 3. Users are encouraged to comment on the choices made by the subcommittee, in the hope of eventually reaching full international harmonization of these terms and concepts.

Key Words

Limit of blank, limit of detection, limit of quantitation, nonparametric statistics

Acknowledgement

This guideline was prepared by NCCLS, as part of a cooperative effort with IFCC to work toward the advancement and dissemination of laboratory standards on a worldwide basis. NCCLS gratefully acknowledges the participation of IFCC in this project.

Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

1 Scope

NCCLS document EP17 specifies recommendations for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of the limits. It also provides guidance for determining lower limits of quantitation based on a laboratory's goals for performance at low-levels. EP17 may be applied to all measurement procedures (even if the reported result is qualitative), most notably those for which the medical decision level is low (i.e., approaching zero).

The intended users of this guideline are clinical laboratory supervisors and manufacturers of *in vitro* diagnostic tests.

2 Introduction

There have been a great number of procedures proposed for establishing the lower limits of detection and quantitation (LoD and LoQ) in laboratory measurement procedures. This document does not intend to review them all, nor does it attempt to find agreement among them. The needs of the clinical laboratory are unique in that the recommended procedure must apply to analytical devices as they are used in the laboratory setting. Usually, this means using the device as it is, often as set up and maintained by a manufacturer or its representatives.

The most commonly recommended statistical protocols for LoD and LoQ, including the ISO protocol 11843, Parts 1 to 4,[1-4] assume that the raw instrument output at low levels can result in negative concentrations. These protocols also assume that the signals for blank samples are normally distributed around some low average value (usually zero). However, many common clinical laboratory instruments report analyte levels in positive numbers only, and basic instrument analytical signals are not retrievable, so the preferred parametric models are not appropriate.

This protocol is intended for use with methods that report in mass or concentration units, and, in particular, in which there is clinical need or interest in very low concentrations (approaching zero). EP17 is intended for all methods, but it was designed specifically for use with methods that report "zero" or positive values only. Manufacturers or developers of methods who have access to the basic instrument response should consider use of ISO 11843-1[1] and either 11843-2[2] or 11843-3,[3] depending on whether or not linear calibration data are available; these documents describe the internationally recognized procedures for determining the LoD. Usually, the expected limits of detection for the EP17 protocol are the same as for the ISO model, but the ISO procedure should return values with lower uncertainty. However, it is important to remember that the use of the parametric ISO procedures requires the validity of the assumptions of normality (Gaussian) at all tested levels, including the blank material.

Proper use of the ISO protocol will also require statistical sophistication that is often beyond the capabilities of most laboratories. The EP17 protocol is intended to be applied and understood without straining the resources available to most clinical laboratories. The procedures are based on the concepts used in conventional LoD procedures, but are practical for use with common clinical laboratory devices.

This protocol requires knowledge of the true (or accepted) levels of the measurand in samples being tested. There is little utility in estimates of precision around an unknown value. It is also important to recognize that modern electronic instruments do not possess continuous functions relating sensor and voltage or current output. The random error measured in the region of a blank may be truly random, but it

