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Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

This document provides guidance for evaluation and documentation of the detection capability of clinical laboratory measurement procedures (ie, limits of blank, detection, and quantitation), for verification of manufacturers' detection capability claims, and for the proper use and interpretation of different detection capability estimates.

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

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Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

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Abstract

Clinical and Laboratory Standards Institute document EP17-A2—*Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition* provides guidance for evaluating the detection capability of clinical laboratory measurement procedures (ie, limits of blank, detection, and quantitation), for verification of manufacturers' detection capability claims, and for the proper use and interpretation of different detection capability estimates. EP17 is intended for use by manufacturers of *in vitro* diagnostic tests, regulatory bodies, and clinical laboratories.

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Foreword

Detection capability is a fundamental performance characteristic of clinical laboratory measurement procedures, most often serving to denote the low-end boundary of a measurement procedure's measuring interval. However, understanding and evaluating detection capability may often be confusing because of the different types of estimates, experimental protocols, and nomenclature used in manufacturers' product claims, as well as within scientific literature throughout the past several decades.

The use of multiple detection capability estimates arises from a need to reflect increasing quantitative certainty within the low-end region of the measuring interval. This ranges from an upper boundary on blank sample measurements (the limit of blank or LoB), through "yes/no" detection of measurand presence (the limit of detection or LoD), up to the minimal measurand amount that can be quantitated reliably with respect to defined accuracy goals (the limit of quantitation or LoQ). Depending on the particular measurement procedure and its application, one, two, or all three of these estimates may be necessary to adequately characterize performance in the low-end region of the measuring interval.

The LoB and LoD are objective statistical constructs that are calculated solely on the basis of the inherent measurement procedure precision and bias. In contrast, the LoQ reflects performance of the measurement procedure vs a preestablished accuracy goal. This is a more subjective value, because the LoQ for a given measurement procedure may vary among different users or applications depending on what are used as the relevant accuracy goals.

The LoD and LoQ are critical when detection of extremely small amounts of a measurand is necessary to define disease states, screen for presence of disease, identify significant exposure, or reveal the presence or absence of substances such as toxins, pollutants, carcinogens, contaminants, infectious agents, and drugs. Knowledge of these estimates also is important for laboratory measurement procedures that measure circulating levels of tumor markers, hormones, infectious disease agents, therapeutic drugs, and other biomarkers for which low results separate subjects into different disease or exposure categories. Even for measurement procedures that report results in qualitative or semiquantitative units, as long as the measurand is a quantity value the developer can use knowledge of the measurement procedure's detection capability to ensure that the measurement procedure design goals were achieved.

Since its original publication in 2004, EP17 has been widely used by manufacturers of *in vitro* diagnostic products to establish product performance claims and by clinical laboratory personnel to verify the claims, and is recognized internationally by regulatory bodies. The present revision builds on the original document by expanding the evaluation protocols to include molecular measurement procedures and providing a more parametric estimate of LoD, as well as by addressing issues of clarity, protocol experimental design requirements, and data analyses. The document title was changed to reflect a broader focus on detection capability as a whole, rather than confining the document to the scope implied by the previous title, *Protocols for Determination of Limits of Detection and Limits of Quantitation.*

Content of this guideline is aligned with International Organization for Standardization document 11843, *Parts 1–5: Capability of detection.*[1-5]

Key Words

Analytical sensitivity, functional sensitivity, limit of blank, limit of detection, limit of quantitation, nonparametric statistics, precision profile, probit

Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

1 Scope

This document provides guidelines for the evaluation and verification of detection capability claims of clinical laboratory measurement procedures (ie, limit of blank [LoB], limit of detection [LoD], and limit of quantitation [LoQ]), as well as for their proper use, documentation, and interpretation. This guidance is suitable both for commercial products as well as laboratory-developed tests. It is particularly important for measurement procedures for which the associated measurand's medical decision level is low (ie, approaching zero).

The intended users of this guideline are manufacturers of *in vitro* diagnostic (IVD) reagents, regulatory bodies, and clinical laboratory personnel.

2 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 features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. The Centers for Disease Control and Prevention address this topic in published guidelines that focus on the daily operations of diagnostic medicine in human and animal medicine while encouraging a culture of safety in the laboratory.[6] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious diseases, refer to CLSI document M29.[7]

3 Terminology

3.1 A Note on Terminology

As a global leader in standardization, CLSI 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. CLSI 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 CLSI, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI's consensus process for development and revision of standards and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

Because of the widespread application of the LoD and LoQ concepts, a variety of terms are in common usage. This document does not attempt to explain or reconcile all of these terms. Terms particular to this document are defined in Section 3.2. However, there are two common terms that have nonstandard usage in the clinical laboratory. To prevent confusion, these terms are discussed in Sections 3.1.1 and 3.1.2.

3.1.1 Nonstandard Use of "Critical Value"

The term "critical value" is defined in ISO 11843-1[1] as the highest result that can reasonably be expected from a blank sample (ie, a sample with concentration at or near zero) for a given error probability α. However, the term is widely used in clinical laboratories for test results that indicate an important medical condition (also sometimes referred to as "alarm value"). In this document, the ISO term is replaced by "LoB."

3.1.2 Nonstandard Use of "Sensitivity"

The term "sensitivity" and its variants "analytical sensitivity" and "functional sensitivity" are not promoted in this document, because of the existence of several conflicting common uses of these terms across multiple technical disciplines. LoD is the preferred term for the detection capability attribute previously associated with analytical sensitivity (ie, signaling presence of a measurand in a sample) because of its more precise definition and common use. Similarly, LoQ is the preferred term for the detection capability attribute previously associated with functional sensitivity (ie, denoting quantitative detection of a measurand in a sample with known measurement accuracy).

3.2 Definitions

accepted reference value – a value that serves as an agreed-upon reference for comparison, and that is derived as a) a theoretical or established value, based on scientific principles; b) an assigned or certified value, based on experimental work of some national or international organization; c) a consensus or certified value, based on collaborative experimental work under the auspices of a scientific or engineering group; and d) when a), b), and c) are not available, the expectation of the (measurable) quantity, ie, the mean of a specified population of measurements (ISO 3534-1).[8]

accuracy – closeness of agreement between a test result and the accepted reference value; **NOTE:** The term "accuracy," when applied to a set of test results, involves a combination of random components and a common systematic error or bias component (ISO 3534-1)[8]; see **trueness.**

analytical sensitivity – quotient of the change in a measurement indication and the corresponding change in a value of the quantity being measured (modified from JCGM 200:2012)[9]; **NOTE 1:** VIM uses the term "sensitivity of a measuring system" (JCGM 200:2012)[9]; **NOTE 2:** The analytical sensitivity of a measuring system is the slope of the calibration curve; **NOTE 3:** Analytical sensitivity should not be used to mean detection limit or quantitation limit, and should not be confused with diagnostic sensitivity (modified from ISO 18113-1).[10]

bias – difference between the expectation of the test results and an accepted reference value (ISO 3534-1)[8]; **NOTE:** Bias is a measure of **trueness.**

blank – sample that does not contain the analyte of interest, or has a concentration at least an order of magnitude less than the lowest level of interest.

censored data – the situation in which measurement results are simply reported as greater than or less than an imposed threshold rather than expressed in quantitative units; **NOTE:** For example, a result is known to be less than a stated limit but the actual result value is not available.

functional sensitivity – the measurand concentration at which precision of a measurement procedure, under stated experimental conditions, meets a stated performance requirement; **NOTE 1:** It is typically determined from a precision profile; **NOTE 2:** The term "limit of quantitation" with stated requirement for accuracy is recommended.

hit rate – proportion of the number of measurement results deemed to indicate presence of a measurand (positive detection result) to the total number of measurement results obtained.

limit of blank (LoB) – highest measurement result that is likely to be observed (with a stated probability [α]) for a blank sample; **NOTE:** LoB is also called "critical value of the net state variable" (ISO 11843-1).[1]

limit of detection (LoD) – measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a measurand in a material is β, given a probability α of falsely claiming its presence (modified from ISO 18113-1)[10]; **NOTE 1:** In quantitative and qualitative molecular measurement procedures, the lowest concentration of analyte that can be consistently detected (typically, in $\geq$ 95% of samples tested under routine clinical laboratory conditions and in a defined type of sample); **NOTE 2:** Also called "lower limit of detection," "minimum detectable concentration" (or value), and "detection limit," and formally referred to as the "minimum detectable value of the net state variable" (ISO 11843-1).[1]

limit of quantitation (LoQ) – lowest amount of a measurand in a material that can be quantitatively determined with stated accuracy (as total error or as independent requirements for bias and precision), under stated experimental conditions (modified from ISO 18113-1).[10]

linearity (of a measuring system) – ability to provide measured quantity values that are directly proportional to the value of the measurand in the sample (Appendix A of ISO 18113-1).[10]

logit – a mathematical transformation function for ratio or percentage values in cumulative logistic distribution probability units.

lower limit of the linear interval (LLLI) – lowest measurand concentration at which the measurement procedure displays a linear relationship with actual measurand content.

lower limit of the measuring interval (LLMI) – the lowest measurand concentration at which all defined performance characteristics of the measurement procedure are met.

measurand – quantity intended to be measured; **NOTE 1:** The specification of a measurand in laboratory medicine requires knowledge of the kind of quantity (eg, mass concentration), a description of the matrix carrying the quantity (eg, blood plasma), and the chemical entities involved (eg, the analyte); **NOTE 2:** The measurand can be a biological activity (modified from ISO 18113-1).[10]

measuring interval – set of values of quantities of the same kind that can be measured by a given measuring instrument or measuring system with specified instrumental uncertainty, under defined conditions; **NOTE:** The measuring interval over which performance characteristics of an *in vitro* diagnostic medical device have been validated has been called the reportable range (modified from ISO 18113-1).[10]

nonparametric – (statistical procedure) a "distribution-free" statistical procedure is also called nonparametric because, unlike a parametric procedure, it does not assume a particular distribution.

parametric – (statistical procedure) one that involves an assumption as to the kind of distribution underlying the data and focuses on estimating a small number of characterizing quantities, called the parameters of the distribution; **NOTE:** For example, a normal (gaussian) distribution is specified by just two parameters, that is, its mean and its SD.

positivity – see **hit rate.**

precision (measurement) – closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions (JCGM 200:2012)[9]; **NOTE 1:** Measurement precision is usually expressed numerically by measures of imprecision, such as SD, variance, or CV under the specified conditions of measurement (JCGM 200:2012)[9]; **NOTE 2:** The "specified conditions" can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement (see ISO 5725-1:1994) (JCGM 200:2012).[9]

precision profile – graphical depiction of the precision of an assay across a measurand concentration interval of interest; **NOTE:** A precision profile is constructed by determining the SD (or CV) of replicate measurements (repeatability, within-laboratory precision, or reproducibility) spanning an analyte concentration interval, albeit without the exact knowledge of the true analyte concentration that is contained in the specimens. When the $CV_{concentration}$ (y-axis) is graphed against the mean values of replicate measurements (x-axis), a precision profile plot is generated. The precision profile is also referred to as the "imprecision profile" by some investigators.

probit – a mathematical transformation function for ratio or percentage values in cumulative normal distribution probability units; **NOTE:** "Probability units" = probits.

probit regression – regression analysis in which the response function Y can have only two responses (ie, detected or not detected) and the predictor variable X is the mean value of replicate measurements; **NOTE:** This technique is widely used to evaluate the limit of detection of molecular measurement procedures.

reference material – material, sufficiently homogeneous and stable regarding one or more properties, with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties (ISO 18113)[10]; **NOTE:** Reference materials with or without assigned quantity values can be used for measurement precision control, whereas only reference materials with assigned quantity values can be used for calibration or measurement trueness control.

total error (TE) – the combined impact of any set of defined precision and bias errors that can affect the accuracy of an analytical result; **NOTE:** TE can be defined as a combination of bias and imprecision according to a specified error model.

trueness – closeness of agreement between the average value obtained from a large series of test results and an accepted reference value; **NOTE:** The measure of trueness is usually expressed in terms of bias (ISO 3534-1).[8]

Type I error (α) – probability of falsely rejecting the null hypothesis that a substance is not present when it is true, ie, a false-positive result.

Type II error (β) – probability of falsely accepting the null hypothesis that a substance is absent, when in fact the substance is present at the designated level, ie, a false-negative result.

verification – provision of objective evidence that a given item fulfills specified requirements (JCGM 200:2012).[9]

3.3 Abbreviations and Acronyms

CEN	Comité Européen de Normalisation (European Committee for Standardization)
CV	coefficient of variation
ID-GC/MS	isotope dilution-gas chromatography/mass spectrometry
IFU	instructions for use

ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
IVD	*in vitro* diagnostic
LLLI	lower limit of the linear interval
LLMI	lower limit of the measuring interval
LoB	limit of blank
LoD	limit of detection
LoQ	limit of quantitation
PCR	polymerase chain reaction
RMS	root mean square
SD	standard deviation
TE	total error
VIM	*Vocabulaire international de métrologie;* International vocabulary of metrology – Basic and general concepts and associated terms (JCGM 200:2012)
WHO	World Health Organization

4 Background

4.1 Overview of Detection Capability

Detection capability is an umbrella term for a set of performance attributes that may be used to characterize measurement accuracy in the low-end region of the measuring interval. These performance attributes are LoB, LoD, and LoQ. They reflect increasing informational content in the measurement procedure's ability to resolve measurand levels, from an upper boundary on expected blank sample measurements (LoB), to simple detection of measurand presence (LoD), to the minimal measurand amount that can be measured with defined accuracy (LoQ).

In describing these attributes, it is necessary to distinguish between the "true" amount of a measurand that is actually present in the sample and individual measurement results. This document uses the terms "actual concentration" to describe what is truly in the sample and "measured concentration" or "result" to describe values that the laboratory will observe when using a particular measurement procedure.

Knowledge of the detection capability for a measurement procedure helps the developer set the low end of its measuring interval. This is done with respect to one of the following terms:

- The lower limit of the measuring interval (LLMI) is the lowest measurand concentration at which all defined performance characteristics of the measurement procedure are met (eg, acceptable bias, imprecision, and linearity).

- The lower limit of the linear interval (LLLI) is the lowest measurand concentration at which the measurement procedure displays a linear relationship with actual measurand content (see CLSI document EP06).[11]

It will always be the case that the LoB is less than the LoD, which is less than or equal to the LoQ. The LLLI may be less than or equal to the LLMI, depending on the developer's functional definition and goals for linearity.

In some special cases, the concepts of LoB, LoD, and LoQ may not be meaningful and the LLMI is set with respect to other criteria. Examples of this are the hemostasis screening tests, prothrombin time and activated partial thromboplastin time, which reflect complex interactions of a large number of proteins, enzymes, and cofactors, with no clear unique measurand. There is no detection limit, per se, and the measuring interval typically is set to reflect instrument processing constraints (ie, time for initial mix) and clinical utility (ie, measurement stopped after some fixed time-out limit).

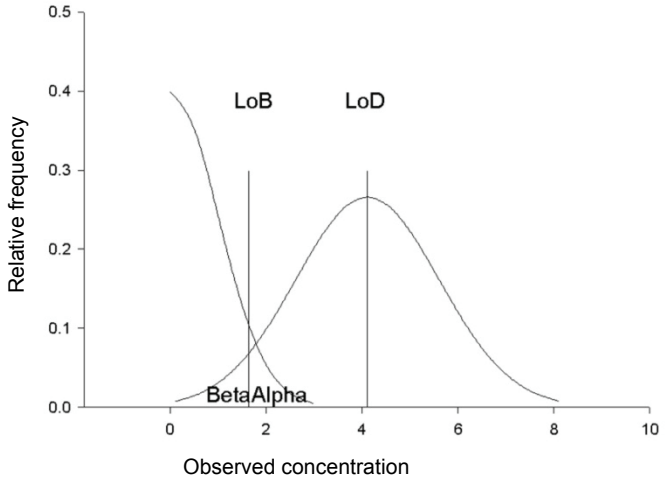
A common complication of assessing detection capability, particularly for clinical laboratory users, is that many instruments automatically suppress reporting of very low and negative measurement results. This can also occur in samples with very low levels of a measurand or for measurement procedures with poor precision. Within this document, this is referred to as "censoring the data." In such cases, true blank samples that would be expected to yield a normal distribution of results about zero may show a truncated distribution (see Figure 1). This artifact can prevent the use of parametric data analysis approaches to calculate detection capability estimates. Developers may be able to access the underlying instrument response signal and convert the signal into a measurement result to overcome the censoring. When developers or laboratorians who must use the standard instrument output results are unable to do so, nonparametric data analysis approaches can be used.

Decades of literature reports across multiple scientific disciplines have yielded an unfortunately wide, confusing, and, at times, contradictory assemblage of terminology and procedures intended for characterizing the detection capability of measurement procedures. Many of these terms and procedures overlap and/or conflict with one another. EP17 will not review most of them, nor attempt to find agreement among them. This document will focus on the unique needs of the IVD industry and clinical laboratories to ensure that the recommended protocols apply to measurement procedures as they are used in those settings.

4.2 Historical Perspectives

The seminal paper by Currie in 1968[12] summarized problems with detection capability nomenclature up to that time and presented a statistically defined approach to the issue, which became well entrenched within analytical chemistry and related fields. Knowledge of the mean and SD from measurement results of blank samples was used to calculate a threshold at which higher values could be interpreted to indicate a positive sample. A sample measurement result at or below this threshold—referred to by Currie as a decision limit—would be taken to represent a "not detected" decision for the presence of a measurand, with an associated probability (α or Type I error) of making a false-positive decision of "detected" when a measurand is truly not present in the sample.

Incorporating knowledge of the SD of the measurement procedure for samples of low measurand content allowed establishment of a second threshold—a detection limit—which accounted for both the probability of making a false-positive decision on a true blank sample (α) and the probability of making a false-negative decision on a sample that truly contains a measurand (β or Type II error). A sample measurement result exceeding this threshold would be taken to represent qualitative detection of measurand presence. Figure 1 illustrates the relationships among the decision and detection thresholds and their associated probability distributions.



Abbreviations: LoB, limit of blank; LoD, limit of detection.

Figure 1. Distributions of Replicates for a Blank Sample (Left Curve) and a Positive Sample of Low Measurand Content (Right Curve). For the blank sample, 95% of its measurement results (taking $\alpha = 0.05$) fall at or below the LoB. With a sample whose measurand content equals the LoD, 95% of its measurement results (taking $\beta = 0.05$) exceed the LoB. The truncated blank sample distribution reflects that some instrument systems suppress measurement results below zero.

To be considered reliable for quantitative analysis, however, the measurement result must be in a region in which its associated SD is a relatively small percentage of its actual value. This required establishment of a third threshold—a determination limit—was based on a subjective decision of where the relative uncertainty of measurement results become acceptable. Although the definition of "acceptable" is open to a developer's interpretation, an early general convention was to set this threshold to the point at which the measurement procedure's SD was 10% of the measurand level (eg, %CV = 10%).[13] There have been a number of exceptions to this initial convention since that time, such as the thyroid-stimulating hormone assay where the conventional target for an acceptable precision limit is often taken as 20 %CV.[14]

This approach, with some clarifications, was used as the basis for detection capability nomenclature by ISO 11843[1-5] and the International Union of Pure and Applied Chemistry (IUPAC).[15] Default levels for the allowable Type 1 and Type II errors were set as $\alpha = \beta = 0.05$. The descriptive nomenclature of Currie was formalized to the following more generalized multidisciplinary terms: critical value of the net state variable (ie, decision limit); minimum detectable value of the net state variable (ie, detection limit); and quantification limit (ie, determination limit). More recently, the terms decision limit, detection limit, and determination limit have been replaced within the IVD industry and clinical laboratories by LoB, LoD, and LoQ, respectively, although the fundamental concepts remain the same.

An older term historically equated with the detection limit in the clinical laboratory is "analytical sensitivity." This value has been typically calculated as two or three SDs above zero, based upon replicate measurement results of a blank sample. Use of this term is problematic for two reasons. First, the preferred definition of analytical sensitivity per IUPAC[15] is the slope of the calibration curve. Secondly, the calculation is based only upon blank sample measurements. There is no consideration of possible precision changes with samples that actually contain the measurand, nor is provision made for the probability of Type II errors. Use of analytical sensitivity—in the detection capability sense—as a performance characteristic for a clinical laboratory measurement procedure is discouraged and should be avoided in IVD device labeling.[10]

A related term is "functional sensitivity." It originally was introduced as a performance characteristic for thyroid-stimulating hormone measurement procedures, denoting the measurand level at which a measurement procedure demonstrated long-term precision of 20 %CV. This attribute was adopted later

for other measurement procedures that have high precision requirements at low measurand levels (eg, troponin, prostate-specific antigen, estradiol) but with wide variability among developers on the type of underlying precision estimates, experimental design for data acquisition, and decision levels for acceptable performance. Functional sensitivity is a form of the LoQ, in which the threshold for results suitable for quantitative analysis is defined solely in terms of a precision requirement. As such, it offers no advantage over the more comprehensive LoQ and promulgates nonstandard usage of the term "sensitivity." Hereafter, functional sensitivity should be superseded by the LoQ in describing measurement procedure performance and for product labeling.[10]

4.3 Current Status

Detection capability of measurement procedures in clinical laboratories today is expressed in terms of the LoB, LoD, and LoQ. It is important to recognize a key difference in how these three limits are established.

The LoB and LoD are statistical constructs based upon variability of the measurement procedure and selection of acceptance probabilities for Type I and II errors. In contrast, establishment of the LoQ depends on the specific acceptance goals used by the developer or user to designate results as acceptable for quantitative analysis with respect to clinical applications of the measurement procedure. These goals may be individual requirements for bias and/or precision, total error (TE) goal (including specification of the particular TE model used), as well as any additional requirements deemed appropriate for the measurement procedure and its particular application. Although it could be possible to set acceptance goals for a measurement procedure so broadly that an LoQ estimate could fall below the statistically based LoD, as a practical convention, the LoQ is restricted to being greater than or equal to LoD because samples with amounts of analyte below the LoD have results of "not detected" more than 5% of the time.

The LoB and LoD are addressed differently for very sensitive tests such as molecular measurement procedures (ie, nucleic acid testing). A hallmark of many such measurement procedures is that there often is no distribution per se of negative sample measurement results (distribution is concentrated at a single point); as such, negative sample results are reported equally as zero. In this case, the LoB is set to zero and negative samples are run simply to confirm performance rather than for use in an LoB calculation. The LoD for such cases is defined as the measurand level at which a specified percentage (usually 95%) of measurement results gives a result that is classified as positive for presence of the measurand, typically based on probit regression (ie, hit rate vs mean value of replicates) data analysis (see CLSI document MM03[16]).

Some measurement procedures may be used with multiple specimen types. It may be appropriate to determine the relevant detection capability estimates for one specimen type and then verify the estimates for the other specimen types. Alternatively, it may be necessary to determine the detection capability estimates for each specimen type, particularly if the measuring interval changes from one specimen type to another.

Given the wide range of measurands, measurement procedures, and the applications with which a clinical laboratory must contend, it is unrealistic to expect that a single experimental protocol could be suitable for the establishment of detection capability estimates in all cases. This document describes several recommended approaches that were selected to cover most cases that arise in clinical laboratories. These include nonparametric, parametric, probit, and precision profile approaches for LoBs and LoDs. A general protocol for LoQ is offered, which can be tailored to meet whatever specific acceptance goals will be applied for a given measurement procedure. It is understood that other approaches may be appropriate, if technically and statistically sound, to meet the specific needs of a particular measurement procedure, its application, and/or performance acceptance requirements.

4.4 Application to Qualitative Measurement Procedures

Estimates of detection capability are important performance characteristics for qualitative measurement procedures in which there is an underlying continuum of instrument signal, yet measurement results are reported as "positive" or "negative" (or their equivalents). For example, enzyme-linked immunosorbent assay measurement procedures use a function of optical density and other instrument responses to distinguish between positive and negative responses. In cases where only instrument signal is available and not measurand concentration, LoB, LoD, and/or LoQ can be seen as potentially useful measurements by the manufacturer, but only for internal quality control purposes.

This information is very useful to document performance capability of such measurement procedures and to monitor consistent performance over time and reagent lots, particularly combined with frequent analysis of a low level control. It is possible to experience an unobserved increase in the LoD of a qualitative test, for example, due to changes in reagents. This can increase the rate of false-negative results, because samples with detectable levels of a measurand may yield results that fall below the cutoff, while samples at higher concentrations may still give correctly positive responses. Consequently, the experimental determination of the LoB and LoD can be important for controlling the quality of many qualitative tests.

Cutoff limits for qualitative measurement procedures typically are based upon either detectability of measurand presence (eg, nucleic acid detection) where this count is potentially quantifiable, or relative position from a clinical threshold where the measurand is not quantifiable outside of the context of this threshold. The first case is effectively the same as an LoB and information in this document may be relevant for manufacturers and users of such devices. Clinically based thresholds, however, can fall at measurand levels significantly above the detection capability for a measurement procedure. Discussion of this class of cutoff limits and the use of detection capability methods for measurement procedures based on such cutoffs is outside the scope of this document.

4.5 General Notes on Sample Selection

The protocols described in subsequent sections of this document rely on testing of blank samples and samples containing low amounts of measurand (low level samples). The following guidance is offered to assist in proper identification and selection of such samples.

Measurements should be acquired from multiple, independent blank and low level samples or pools of samples in order to account for matrix variability among samples. At least four individual, unique, and natural patient samples of each type (blank and low level) should be used in the study. To the extent possible, blank and low level samples should reflect performance consistent with native patient samples. It is acceptable to dilute or spike samples in order to provide low level samples at desired measurand levels, providing that such samples perform similarly to native patient samples in the measurement procedure.

Blank samples represent patient samples with no measurand content. Appropriate examples include the use of drug-free serum samples for a drug measurement procedure or virus/bacteria–free samples for molecular diagnostics measurement procedures. For endogenous measurands, suitable blank samples might be constructed by stripping the measurand from native patient samples using techniques such as precipitation by an antibody, enzymatic degradation, and adsorption to charcoal, among others, although it is understood that such manipulation of samples may risk altering other constituents that might be important to the measurement procedure (eg, binding proteins). Blank samples for hormone measurands may come from diseased subjects or subjects with suppressed measurand levels due to pharmacological treatment, while samples from nondiseased subjects may be appropriate for tumor marker measurands. Sample diluents and similar matrixes may be viable substitutes for true blank patient samples. Analyte-free samples such as saline, water, or protein solutions may be used as blank samples in cases in which

they can be demonstrated to perform similarly to patient samples through linearity, recovery, and/or other testing, as appropriate.

If a residual level of a measurand is unavoidable, it should be at least an order of magnitude below the limit of the measuring range for the measurement procedure. Because artificial and/or spiked samples may behave differently than native samples, the reader is advised to review CLSI documents EP07[17] and EP14[18] before using samples that are not natural patient samples.

4.6 General Notes on Data Collection and Review

Most of the evaluation protocols described in this document involve testing that is conducted across multiple combinations of different experimental factors. There are several ways to schedule such testing (eg, test all reagent lots across all instrument systems each day, test individual reagent lots and/or instrument systems sequentially). The developer is free to create a suitable processing plan that is consistent with the constraints of the measurement procedure and available testing resources.

It may be possible to obtain measurement results without censoring by the instrument system. In some cases this may be done by overriding the standard way that measurement results are reported by the instrument system. In other cases, it may be possible to obtain the raw instrument response signals and use an offline routine to convert them into measurement results. In this scenario, the only change is avoidance of censoring by a software filter used by the instrument for reporting quantitative results. There are no changes to the underlying calibration or sample processing steps from routine user operation.

The evaluation protocols described in this document require acquisition of experimental testing results. Ideally, data should be reviewed as soon as possible after completion of a testing run to check for possible processing errors or missing results. This is the best time to identify potential outliers and assignable causes for them (eg, mislabeled samples, insufficient sample volume, presence of clots, data transcription). Visual inspection and statistical analyses (eg, Grubbs test[19]) may aid in assessment. Outliers arising from such assignable causes—aside from analytical errors of the measurement procedure itself—may be retested and substituted into the data, ideally on the same testing day. Any such retests must be documented, along with the original test results.

When all results are in hand for a study, the full dataset should be inspected for consistency and possible presence of outlier results. This may be done by visual inspection after plotting the data and/or through use of software tools to identify suspected atypical results. It is assumed that the data were reviewed for atypical results due to assignable causes during the collection phase and corrected as appropriate. If any apparent extreme results remain in the final datasets that could not be explained, it may be warranted to consider the use of appropriate statistical analyses for classification of such results as statistical outliers. One should consult with a statistician to ensure that any such analysis is appropriate, while keeping in mind the inherent risk in eliminating an outlier result that might actually reflect the extreme of typical performance. Elimination of such statistical outliers from a dataset also may have implications for review of the measurement procedure by regulatory bodies. Data analysis should be performed with the inclusion of all results as well as after exclusion of deemed statistical outliers, in order to determine their impact.

5 Protocols for Evaluation of the Limit of Blank and Limit of Detection

5.1 Introduction

This chapter provides a set of protocols that can be used to estimate the LoBs and/or LoDs, along with suggestions on selecting which one may be best suited to meet the needs of a particular type of measurement procedure. The protocols and the detection capability limits that they provide are:

	LoB	LoD
• Classical (original EP17-A approach)	✓	✓
• Precision profile	no	✓
• Probit	no	✓

These protocols are described in the following subsections in terms of underlying experimental design, sample requirements, procedural steps, and data analysis. It should be understood that the protocol descriptions are based on the minimal acceptable experimental design requirements. Depending on the particular measurement procedure and desired statistical rigor of the resulting estimates, it may be appropriate to augment the specific design factors in the experimental design, the number of levels of some factors, and/or the number of replicate measurements to be acquired.

Please note that each of these protocols has two paths for data analysis, which are split by the number of reagent lots used in the study. The first path is for studies where two or three reagent lots are used. For these, detection capability is estimated independently for each reagent lot and the maximum value is reported for the overall study. The second path is for studies where four or more reagent lots are used. For these, the data from all reagent lots are combined. Detection capability is estimated for the combined dataset and reported as the result of the overall study. The reason for the different paths is that studies with only two or three reagent lots could be unduly influenced by significant reagent lot-to-lot variability. Such variability would be expected to be more smoothed out in designs using four or more reagent lots, allowing for the use of simplified calculations.

Other approaches may be appropriate to provide estimates of the LoBs and LoDs in order to meet the particular needs of a measurement procedure, its application, and/or performance acceptance requirements. In these cases, the onus is on the developer to ensure and document that such an alternate approach is both technically sound and statistically valid.

5.2 Selection of Experimental Protocol

Each of the recommended protocols has associated strengths and weaknesses that make it better suited for use with certain types of measurement procedures. This section gives a brief discussion of the protocols, along with some examples for which they may be best suited.

- **Classical Approach**

  This is the original approach from the previous version of this document, which has been widely used for many chemistry and immunochemical measurement procedures. It uses measurements made on both a set of blank samples and a set of low level samples containing a measurand targeted around the assumed LoD. Depending upon the distributions of the blank and low level sample results, a nonparametric or—more rarely—a parametric data analysis option is selected to calculate the LoB and LoD estimates. An underlying assumption is that variability of measurement results is reasonably consistent across the low level samples. A variant design (trial and error) is described for cases in which this assumption is not met.

- **Precision Profile Approach**

  The precision profile approach is useful when the variability of measurement results changes significantly in the region of the assumed LoD. It also may be useful in cases in which the developer wishes to make use of precision data that were acquired over a wider concentration interval than typically used for detection capability studies. Implementation of this approach assumes that variability of measurement results vs the mean measurand concentration can be fitted adequately with a precision profile model.

- **Probit Approach**

  Molecular measurement procedures (eg, for infectious diseases, nucleic acid testing) differ from typical measurement procedures because all blank or negative sample results normally are reported as negative. The false-positive rate is much lower than 5% (typically below 0.5%), and the LoB, typically, is taken to be zero. The LoD is calculated from a probit regression model as the measurand concentration at which, with a predefined probability (usually 95%), measurement results yield a positive classification.

Developers should assess the assumptions and constraints described above to select a suitable protocol for their particular measurement procedures. Other protocols may be suitable for a particular measurement procedure or application. In such cases, it may be wise to consult with appropriate regulatory bodies on the need for any such alternate protocol, as well as its technical and statistical merits.

5.3 Classical Approach

The classical approach expands on the protocol described in the previous version of this document. For a given measurement procedure, the developer obtains a series of replicate results on each of a set of blank samples and on a set of low level samples. A requirement for this approach is that the variability of measurement results is relatively consistent across the low level samples.

The results from the blank samples are used to determine a threshold—the LoB—above which results from true blank samples would be very rare. If a true blank sample gave a measurement result exceeding this threshold and a developer concluded that it was from a positive sample, he or she committed an error referred to as a Type I error. The associated error risk is denoted by α. For a typical 95% LoB threshold, the Type I error risk is 5% ($\alpha = 0.05$).

Conversely, it is possible that the measurement result from a true low level sample could fall below the LoB threshold and be indistinguishable from the population of blank measurement results. If a developer concludes that a measurand is not present in this case, he or she commits an error known as a Type II error, with an associated error risk denoted by β. The LoD reflects the measurand level at which the likelihood of a true low level sample giving a false-negative result (ie, measurement result below the LoB) equals the specified Type II error risk.

Nominal values for Type I and II error risks are $\alpha = \beta = 0.05$. Developers may set α and β as appropriate, however, depending on the relative allowable error risks for a particular measurement procedure. Statements of the LoB and LoD should include the associated α and β error risks.

This protocol includes both nonparametric and parametric data analysis options to calculate the LoB and LoD from the blank and low level samples' measurement results. Selection between them is guided by the distributional shape of measurement results from the blank samples. A worked example following the classical approach is provided in Appendix A.

5.3.1 Experimental Design

5.3.1.1 Protocol Requirements

The experimental design consists of replicate measurements on blank and low level samples using multiple reagent lots across multiple days, with a single instrument. An LoB estimate is calculated for each reagent lot. The maximum observed LoB across all reagent lots (for the case of two or three reagent lots) or the LoB estimate for combined data of all reagent lots (for the case of four or more reagent lots) is taken as the reported value for the measurement procedure and used to calculate LoD estimates. The maximum observed LoD across all reagent lots (for the case of two or three reagent lots) or the LoD

estimate for combined data of all reagent lots (for the case four or more reagent lots) is taken as the LoD estimate value for the measurement procedure.

The minimal experimental design is:

- Two reagent lots
- One instrument system
- Three days
- Four blank samples
- Four low measurand content (positive) samples
- Two replicates per sample (for each reagent lot, day, and instrument system combination)
- 60 total blank replicates per reagent lot (across all blank samples, days, and instrument systems)
- 60 total low level sample replicates per reagent lot (across all low level samples, days, and instrument systems)

The minimal design described above (ie, one instrument system, three days, four samples, two replicates) does not yield the necessary 60 total replicates of blank or low level samples per reagent lot. It is necessary for the developer to increase one or more design factors to provide a sufficient number of measurement results. The selection of which factors to increase depends on the particular measurement procedure and available resources for testing. The developer also may wish to add more factors (eg, calibrator lot, calibration cycle, operator) and/or to increase the number of replicates beyond the minimum in order to increase the rigor of the resulting detection capability estimates.

The minimum number of 60 replicates for the blank and low level samples represents a reasonable compromise between uncertainty of the detection capability estimates and the cost of performing the study. Further information on the relationship between the number of replicates and uncertainty of the estimates is described by Linnet and Kondratovich.[20]

It is not necessary to have the same number of blank and low level samples, as long as the minimum number of requirements is met.

5.3.1.2 Sample Selection

Follow the guidance in Section 4.5 regarding identification and selection of appropriate blank and low level samples.

Low level samples represent patient samples in which the measurand concentration is in the approximate region of the assumed LoD. This is usually estimated as a range of one to five times the estimated LoB. Samples of greater measurand concentration may be suitable as long as their associated measurement procedure variabilities are similar to that at lower concentrations. A rough estimate of the LoB, to assess the range of measurand concentration to be used for evaluation of the LoD, may be calculated as the maximum value of 20 replicate measurements of a single blank sample.

5.3.2 Experimental Steps

1. Decide on the experimental design factors and number of levels for each factor to be used (see minimal requirements in Section 5.3.1.1), as well as a processing plan to test the factors with the specific measurement procedure.

2. Identify and prepare sufficient aliquots of all blank and low level samples to complete the planned testing. Ensure that extra aliquots are provided to accommodate possible testing errors or processing upsets.

3. Each testing day, process the designated number of replicate tests for each sample according to the processing plan.

4. Review the measurement results each testing day to check for possible processing errors or missing results. Identify potential outliers and assign causes for them (eg, short sample, instrument processing error, sample identification mix-up). Outliers arising from such assignable causes—aside from analytical errors of the measurement procedure itself—may be retested and substituted into the data, ideally on the same testing day. Any such retests must be documented, along with the original test results. Presence of more than five such outliers identified with assignable causes across all blank or all low level sample results from any one reagent lot is reason to reject and repeat the study for that reagent lot.

5. Ensure that sufficient measurement results are available at the end of testing to start data analysis. A minimum of 60 total blank and 60 total low level sample results are required per reagent lot.

5.3.3 Data Analysis

The analysis process uses the following sequence of steps to provide the final LoB and LoD estimates.

1. Select the α and β values to use for the LoB and LoD estimates (typically $\alpha = \beta = 0.05$).

2. Examine the combined distribution of blank sample results across all reagent lots to determine if the requirements described in Section 5.3.3.1 were met, to allow use of the parametric option.

3. Select the data analysis option to use; ie, nonparametric or parametric.

4. Calculate the LoB for the overall study, based upon the number of reagent lots used in the study.

5.3.3.1 Calculation of Limit of Blank

Selection of a data analysis option to calculate the LoB depends on the distribution shape of the blank sample measurement results. The nonparametric option makes no distributional assumptions and may be used for any dataset. It is particularly appropriate if the blank sample measurement results show evidence of censoring or a significant non-normal distribution. Use of the parametric option requires that blank sample measurement results are reasonably well described by a normal distribution, but that the data may contain outlying values. It may be useful to perform a statistical test to assess the normality of the data to aid in deciding which data analysis approach to use. In practice, the overwhelming number of cases will use the nonparametric option.

Do not change values of results with negative values. These would be expected from a distribution of measurements for a sample at zero measurand content using an instrument system without censoring (eg, distribution of results for a phenobarbital measurement procedure with drug-free patient samples). If the results are collected from an instrument system in which censoring is active, negative results are unlikely.

Nonparametric Option[20]

Perform Steps 1–4 independently on the data for each reagent lot if there are two or three reagent lots used in the study. Combine all data across reagent lots and perform Steps 1–4 on the combined dataset if there are four or more reagent lots used in the study.

1. Sort all B blank sample measurement results from lowest to highest, where B is the total number of results in the dataset: $X_{(1)}, X_{(2)}, \ldots, X_{(B)}$.

2. Calculate the pth percentile for the distribution of blank sample results (Pct_B) corresponding to the desired α (Type I error) risk probability (eg, $\alpha = 0.05$ yields $p = 0.95$).

3. Calculate the rank position corresponding to the Pct_B percentile as:

$$\text{Rank Position} = 0.5 + B \bullet 0.95 \quad (1)$$

4. LoB is taken as the value of the measurement result at the rank position calculated in Step 3. If the calculated rank position is a noninteger, LoB is calculated by interpolation from measurement results with bracketing rank values.

   For example, if $B = 60$, then Rank Position = 0.5 + 60 • 0.95 = 57.5. This requires the LoB to be calculated by interpolating from the surrounding measurement results of ranks 57 and 58.

   $\text{LoB} = X_{(57)} + 0.5(X_{(58)} - X_{(57)}) = 0.5(X_{(57)} + X_{(58)})$.
   Similarly, if $B = 65$, Rank Position $= 0.5 + 65 \bullet 0.95 = 62.25$ and $\text{LoB} = X_{(62)} + 0.25(X_{(63)} - X_{(62)})$.

5. If there are two or three reagent lots, then the LoB of the measurement procedure is a maximal value of the LoBs obtained for each reagent lot. If there are four or more reagent lots, then the LoB calculated from Steps 1–4 above for the combined data is the LoB of the measurement procedure. The LoB of the measurement procedure is the value taken forward into LoD calculations (see Section 5.3.3.2).

Parametric Option

Perform Steps 1–2 independently on the data for each reagent lot if there are two or three reagent lots used in the study. Combine all data across reagent lots and perform Steps 1–2 on the combined dataset if there are four or more reagent lots used in the study.

1. Calculate the mean (M_B) and SD (SD_B) of all blank results in the dataset.

2. Calculate the LoB as:

$$\text{LoB} = M_B + c_p SD_B \quad (2)$$

$$c_p = \frac{1.645}{1 - \left(\frac{1}{4(B - K)} \right)} \quad (3)$$

   where c_p is a multiplier to give the 95th percentile of a normal distribution (corrected for use of the biased observed SD estimate instead of the true but unknown population SD), B = total number of blank results in the dataset, and K = number of blank samples.

NOTE 1: The value 1.645 represents the 95th percentile from the normal distribution for $\alpha = 0.05$. If a different α value is chosen as the basis for an LoB estimate, this multiplier will need to change accordingly.

NOTE 2: The (B − K) term in the denominator represents the degrees of freedom of the estimated SD_B.

3. If there are two or three reagent lots, then the LoB of the measurement procedure is a maximal value of the LoBs obtained for each reagent lot. If there are four or more reagent lots, then the LoB calculated from Steps 1–2 above for the combined data is the LoB of the measurement procedure. The LoB of the measurement procedure is the value taken forward into LoD calculations (see Section 5.3.3.2).

5.3.3.2 Calculation of Limit of Detection

Calculation of the LoD follows a parametric analysis regardless of whether the LoB was determined by the nonparametric or parametric analysis option. It is assumed that variability of measurement results is relatively consistent across the low level samples. This may be confirmed using a statistical method such as Cochran's test.[19] If this test fails, the developer needs to investigate the root cause. It may reflect instability of the reaction, an inappropriately wide measurand range across the chosen low level samples, or other causes. In such cases, another approach may be required, such as the trial and error experimental design (see the LoD Variant Approach: Nonparametric Analysis section below) or the precision profile approach (see Section 5.4), or, ultimately, the study may need to be repeated using a more appropriate sample selection to improve heterogeneity of measurement procedure variability across the samples.

Parametric Analysis

If appropriate, apply a mathematical transformation to yield a normal distribution of the low level samples' measurement results before starting the calculations below.

Perform Steps 1–3 independently on the data for each reagent lot if there are two or three reagent lots used in the study. Combine all data across reagent lots and perform Steps 1–3 on the combined dataset if there are four or more reagent lots used in the study.

1. Calculate the SD for each of the low level samples in the dataset.

2. Calculate the pooled SD_L across all J low level samples as:

$$SD_L = \sqrt{\frac{\sum_{i=1}^{J}(n_i - 1)SD_i^2}{\sum_{i=1}^{J}(n_i - 1)}} \quad (4)$$

where:
SD_i = SD of all results for the ith low level sample
n_i = the number of results for the ith low level sample
J = number of low level samples

3. Calculate the LoD as:

$$\text{LoD} = \text{LoB} + c_p \text{SD}_\text{L} \quad (5)$$

$$c_p = \frac{1.645}{1 - \left(\frac{1}{4(L - J)} \right)} \quad (6)$$

where c_p is a multiplier to give the 95th percentile of a normal distribution (corrected for use of the biased observed SD estimate instead of the true but unknown population SD), L = total number of all low level sample results across all reagent lots, J = number of low level samples, and LoB is a limit of blank for the measurement procedure (calculated per Section 5.3.3.1).

NOTE 1: The value 1.645 represents the 95th percentile from the normal distribution for $\beta = 0.05$. If a different β value is chosen as the basis for an LoD estimate, this multiplier will need to change accordingly.

NOTE 2: The (L − J) term in the denominator represents the degrees of freedom of the estimated SD_L.

4. If there are two or three reagent lots, then the LoD of the measurement procedure is a maximal value of the LoDs obtained for each reagent lot. If there are four or more reagent lots, then the LoD calculated from Steps 1–3 above for the combined data is the LoD of the measurement procedure.

LoD Variant Approach: Nonparametric Analysis

If the variability of measurement results is not closely consistent with a normal distribution and it is not possible to transform the low level sample results to a near normal distribution, a nonparametric approach may be used to identify the LoD. The same experimental design, requirements, and testing procedure apply as outlined in Sections 5.1 through 5.3.3.1, except for selection of the low level samples. For the nonparametric approach, an *a priori* decision is made as to the expected LoD measurand concentration and all of the low level samples should be targeted to this value.

Following data collection, the LoB for the measurement procedure is determined per Section 5.3.3.1. All measurement results across the low level samples for a given reagent lot are combined into a single distribution, and then the percentage of individual results falling below the LoB value is computed. If the percentage is less than the desired Type II (β) error, the LoD for that reagent lot is taken as the median of the combined low level sample result distribution. If there are two or three reagent lots, then the LoD of the measurement procedure is a maximal value of the LoDs obtained for each reagent lot. If there are four or more reagent lots, then the LoD is calculated for the combined data across all reagent lots and is the LoD of the measurement procedure.

For example, the typical $\beta = 0.05$ Type II error requirement means that no more than 5% of the low level sample distribution results fall below the LoB. If the outcome from one or more reagent lots fails to meet the Type II error requirement, the study is repeated with a new set of low level samples at a higher measurand concentration. It is not necessary to repeat the LoB portion of the study. Testing continues until a trial measurand concentration is reached in which the combined distribution of low level sample results for each reagent lot (two or three lots) or across all reagent lots (four or more lots) meets the Type II error requirement. This measurand concentration is taken as the LoD for the measurement procedure.

5.4 Precision Profile Approach

The precision profile[21] approach may be useful in situations in which the variability of measurement results follows a relatively normal distribution but changes in magnitude within the region of the expected LoD. It may also be useful when the developer does not have a clear initial estimate of the LoD and wishes to incorporate a wider interval of measurand concentrations than might be practical using the classical approach. A precision profile approach also lends itself to integrating an LoD study within precision evaluation studies, such as within CLSI document EP05,[22] as well as data mining of collected results from a group of such precision studies incorporating a variety of reagent and calibrator lots, instrument systems, operators, etc.

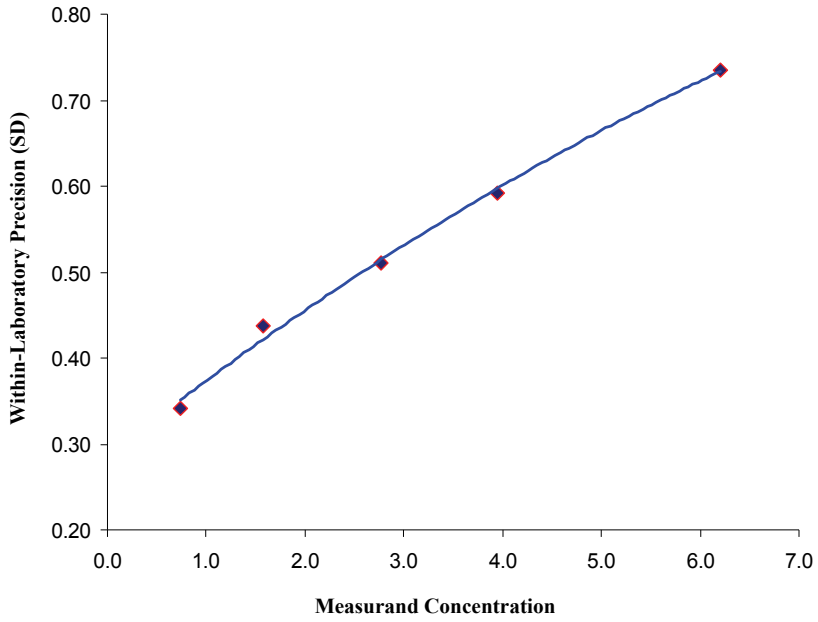


Figure 2. Example Precision Profile. A set of patient sample pools was tested over 20 days using the protocol within CLSI document EP05[22] to determine estimates of within-laboratory precision. The resulting SD estimates were plotted against their respective mean measurand concentrations, then fit by a second-order polynomial model.

A summary of this approach starts with selection of patient samples that bracket the assumed LoD and span a desired measurand concentration interval, typically broader than what is used for the classical approach. Precision studies are conducted to yield estimates of within-laboratory precision for each of these samples. A precision profile is made by plotting within-laboratory precision (as variance, SD, or %CV) on the y-axis vs the respective mean measurand concentrations on the x-axis, as shown in Figure 2. The data are fit with a suitable model, which is then used recursively to compute trial LoD values from the predicted SDs. When a trial LoD value matches the measurand concentration used to generate the predicted SD, that value is taken as the LoD estimate for the measurement procedure. See Appendix B for a worked example of evaluating the LoD using this approach.

Issues from data censoring affect the precision profile approach in the same way as for the classical approach. As such, it may be best to estimate the LoB through the classical approach with nonparametric data analysis, and then use the precision profile approach to estimate the LoD. Alternatively, it may be possible to incorporate appropriate blank samples as part of the precision profile experiment and use specialized data analysis tools (eg, truncated normal distribution, L1-norm method) to extract the relevant statistics for an LoB estimate. Such treatments are beyond the scope of the approach presented in this section and will not be further described in this document.

5.4.1 Experimental Design

5.4.1.1 Protocol Requirements

The experimental design is used to process replicates of sets of patient samples or their equivalents according to a technically and statistically valid precision protocol (eg, CLSI documents EP05[22] and EP15[23]) to yield estimates of within-laboratory precision. An LoD estimate is calculated for each reagent lot. The maximum observed LoD across all reagent lots (for the case of two or three reagent lots) or the LoD estimate for combined data of all reagent lots (for the case of four or more reagent lots) is taken as the reported value for the measurement procedure.

The minimal experimental design is:

- Two reagent lots
- One instrument system
- Five days
- Five samples
- Five replicates per sample
- 40 replicates per sample (across all testing days and instrument systems) per reagent lot

This list describes the minimal experimental design requirements. Multiple ways exist to structure experimental testing. Separate precision studies may be performed for specific reagent lot and instrument system combinations, or a single study could be run incorporating all levels of all factors together. A developer also may wish to expand the design to add more levels of each experimental factor and/or add more factors (eg, instrument systems, calibrator lot, calibration cycle, operator) depending on the nature of the measurement procedure and the desired rigor of the resulting detection capability estimates.

The minimal design described above (ie, five days and five replicates per sample) does not yield the necessary 40 total replicates per sample per reagent lot. It is necessary for the developer to increase one or more design factors to provide a sufficient number of measurement results. The selection of which factors to increase depends on the particular measurement procedure and available resources for testing.

It is not required that all precision studies be run simultaneously. Results may be collected from separate studies performed over time, as is often done during development of measurement procedures for which multiple reagent lots are only available on a staggered production schedule. The developer is cautioned, however, that use of such a data mining approach for data acquisition might negatively impact cohesiveness of the results, significantly increasing scatter about the precision profile and difficulty in obtaining a satisfactory fit of the precision model.

5.4.1.2 Sample Selection

Follow the guidance in Section 4.5 regarding identification and selection of appropriate blank and low level samples. It is usually good practice to select samples with measurand concentrations in the low-end region of the measuring interval (ie, one time to 10 times the assumed LoB) in order to avoid undue leverage of elevated samples on the curve fit. This effect can be readily seen in the precision profile plots in which the impact of elevated samples may cause the curve fit to over- or undershoot the points in the low-end region of interest. It is not often the case that a precision profile can be constructed that spans the entire measuring interval for a measurement procedure with adequate fit in the low-end region. A prudent middle ground may be to include some samples somewhat above the low-end region, then eliminate them stepwise by decreasing measurand concentration until the quality of fit is acceptable by visual or other criteria.

Ensure that sufficient sample volume is available to last through the full study, while allowing extra volume in case of processing errors or other procedural problems. A generally good practice is to aliquot samples into suitable volumes for each test point and freeze them to ensure stable, consistent samples—assuming it is known that the analyte is stable under such conditions. Aliquots are withdrawn from storage before each test point and allowed to warm/mix by a defined protocol.

5.4.1.3 Precision Model

Success with the precision profile approach is greatly dependent on the particular model used to fit the precision estimate vs concentration data and the resulting quality of the fit. Selection of a suitable model depends upon the sample measurand concentration range tested. Over a narrow enough range, even a linear model may suffice. Although several potential models are described in the literature, three of the more widely used ones in clinical literature are the linear model, a quadratic model, and the Sadler precision profile model,[24] which is described by:

$$SD_{WL} = \left(B_1 + B_2 X\right)^{B_3} \quad (7)$$

where SD_{WL} represents within-laboratory precision, X is the associated measurand concentration, and B_1–B_3 are parameters to be estimated in the model fit process.

In addition to the form of the precision model, a developer also needs to decide on suitable criteria for goodness of fit to ensure that the model will provide meaningful results over the desired measurand concentration range. These criteria may be numerical (eg, absolute or relative error of predicted vs actual precision values) and/or visual (eg, observed curve should go smoothly through underlying data). Consultation with a statistician may be helpful for selection of a suitable model and fit criteria.

5.4.2 Experimental Steps

This section assumes that the precision profile approach is used to estimate the LoD and that the LoB is provided from the classical approach as described above in Section 5.3.

1. Decide on the experimental design factors and number of levels for each factor to be represented in the precision studies (see minimal requirements in Section 5.4.1.1), as well as the precision study protocol to use and timing to perform each study. The same protocol should be used for all precision studies.

2. Identify and prepare sufficient aliquots of all samples to complete the planned testing. Ensure that extra aliquots are provided to accommodate possible testing errors or processing upsets.

3. At each testing point, process the appropriate number of replicate tests per sample according to the precision study protocol.

4. Review the measurement results each testing day to check for possible processing errors or missing results. Identify potential outliers and assignable causes for them. Outliers arising from such assignable causes—aside from analytical errors of the measurement procedure itself—may be retested and substituted into the data, ideally on the same testing day. Any such retests must be documented, along with the original test results. Presence of more than five such outliers identified with assignable causes across all blank or all low level sample results from any one reagent lot is reason to reject and repeat the study for that reagent lot.

5.4.3 Data Analysis

The analysis process uses the following sequence of steps to provide the final LoD estimate:

1. Fit a model to the precision profile.

2. Assess quality of the model fit to ensure its applicability for further analysis.

3. Starting at the LoB measurand concentration, then increasing, iteratively calculate within-laboratory precision SD estimates using the precision profile model and their corresponding trial LoD estimates.

4. Take as the LoD the measurand concentration at which the predicted SD leads to an LoD estimate that equals the measurand concentration (within desired numerical precision).

5.4.3.1 Calculation of Limit of Blank

Follow the procedure in Section 5.3.3.1 to generate an LoB estimate using the parametric or nonparametric data analysis option, as appropriate. Alternatively, derive the LoB estimate from suitable analysis of precision results from blank per the precision profile approach (see discussion and reference in Section 5.4).

5.4.3.2 Calculation of Limit of Detection

Perform Steps 1–4 independently on the data for each reagent lot if there are two or three reagent lots used in the study. Combine all data across reagent lots and perform Steps 1–4 on the combined dataset if there are four or more reagent lots used in the study.

1. Create a precision plot of within-laboratory precision (as variance, SD, or %CV, per requirements of the fitting model to be used) on the y-axis vs measurand concentration on the x-axis, based on the results of all precision studies included in the LoD plan.

2. Fit the precision profile data with the desired model. Assess its goodness of fit per the appropriate acceptance criteria. If the model fit is not deemed acceptable, consider expressing precision in a different format (eg, variance, SD, or %CV) and refit the model. Other options are to refit using a shortened data range (eg, eliminate the highest and/or lowest value) or use an alternate model.

   NOTE: One must be careful if electing to shorten the data range, and be sure to retain at least P + 1 data points, where P is the number of fit parameters in the model (eg, P = 3 for the Sadler model shown in Section 5.4.1.3; therefore, at least four data points must be kept). Another concern is that the final range must bracket the LoD to avoid edge effects or other data analysis artifacts. This may require redoing the data analysis if the final LoD estimate falls outside of a trimmed data range.

3. Start with the LoB measurand concentration (because, by definition, the LoD cannot be less than the LoB), compute the predicted within-laboratory precision (SD_{WL}) from the precision profile model, and use it to compute a trial LoD value. If the precision profile model uses %CV as the response variable, it will be necessary to convert the %CV values into the respective SD_{WL} values before using the following equations:

$$\text{LoD} = \text{LoB} + c_p SD_{WL} \quad (8)$$

$$c_p = \frac{1.645}{1-\left(\frac{1}{4(N_{TOT}-K)}\right)} \quad (9)$$

where LoB comes from a separate study or analysis (see Section 5.4.3.1); c_p is a multiplier to give the 95th percentile of a normal distribution, corrected for use of the biased observed SD estimate instead of the true (but unknown) population SD; and N_{TOT} is the total number of measurement results over all K precision studies used to construct the precision profile.

NOTE 1: The value 1.645 represents the 95th percentile from the normal distribution for $\alpha = 0.05$. If a different α value is chosen as the basis for an LoB estimate, this multiplier will need to change accordingly.

NOTE 2: For all practical precision profile studies, F will be large enough that the correction factor is insignificant, leaving $c_p = 1.645$.

NOTE 3: Trial LoD values should be expressed with the same numerical precision (ie, number of decimal places) as the measurand concentrations in the precision profile dataset.

4. Sequentially increase the measurand concentration, then solve for the predicted within-laboratory precision SD_{WL} and associated trial LoD estimate per equations 7–9. Continue the process until a measurand concentration is reached that yields a matching trial LoD estimate, within the desired numerical precision. This value is taken as the LoD for the dataset.

5. If there are two or three reagent lots, then the LoD of the measurement procedure is a maximal value of the LoDs obtained for each reagent lot. If there are four or more reagent lots, then the LoD calculated from Steps 1–4 above for the combined data is the LoD of the measurement procedure.

The iterative approach applied in these calculations is necessary when the model used to fit the precision profile data does not have an explicit analytical solution. There are particular models, however, for which an analytical solution is readily available and may be used. An example of this is provided below for a linear precision profile model, using the LoD definition in equation 8.

Linear Model

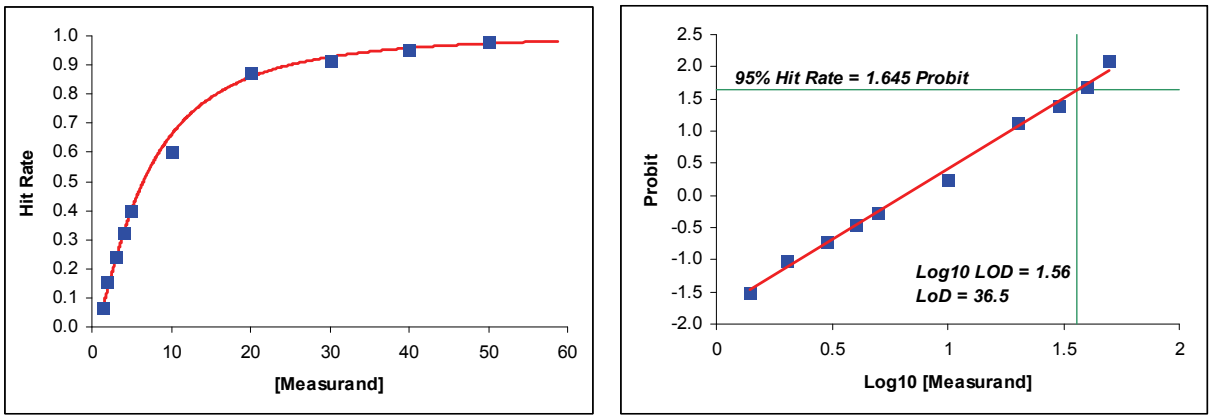
$$SD_{WL} = C_0 + C_1 X \quad (10)$$

$$LoD = \frac{LoB + c_p C_0}{(1 - c_p C_1)} \quad (11)$$

5.5 Probit Approach

The probit approach is useful to evaluate the LoD for measurement procedures whose detection capabilities are assessed in terms of proportion (ie, number of positive results with respect to the total number of replicate tests) and it can also be used for direct measurand quantitations when the LoB is used for defining "positive" results. Although initially applied to assessment of pesticide efficacy,[25] the use of probit analysis has since expanded across multiple disciplines, such as toxicology, microbiology, pharmacology, and virology, particularly with the advent of PCR techniques.[26] A related approach based on the logit function is very similar, differing in terms of the underlying probability function (logit has slightly flatter tails than the probit function). Although either could be used in similar applications, this document will focus on the probit approach.

Typical use of the probit approach follows a limiting dilution dose-response protocol. A set of serial dilutions are made from a starting sample of known measurand content. These dilutions are tested in replicate by the measurement procedure in which results are judged to be one of two outcomes: detected or not detected. For each dilution, a ratio (the hit rate) is computed as the number of replicates with a "detected" outcome per the total number of replicates tested. These hit rates are converted mathematically into cumulative normal probability units (probits) and fitted by a regression model vs their respective measurand concentrations. Finally, the regression model is used to compute the measurand concentration corresponding to a predefined hit rate (eg, 0.95), which is then taken as the LoD. An illustration of the analysis is given in Figure 3, with a worked example in Appendix C.



Abbreviation: LoD, limit of detection.

Figure 3. Probit Analysis. These plots illustrate hypothetical experimental results (left hand plot) and regression analysis to determine the LoD for a molecular measurement procedure by the probit approach (right hand plot).

Within the clinical laboratory, probit analysis is particularly important for measurement procedures based upon molecular techniques such as those to detect infectious agents (eg, hepatitis C virus, hepatitis B virus, human immunodeficiency virus) and other nucleic acid tests using PCR technology for target amplification and detection. Such measurement procedures have essentially no distribution of measurement results for negative samples, because all such results are typically reported as zero. In these situations, the 95th percentile of test results on blank samples equals zero, and LoB is set to zero by definition. Otherwise, the nonparametric approach described in Section 5.3.3.1 may be used to establish an LoB for the measurement procedure.

It is important to include representative samples of all relevant genotypes when evaluating the detection capability of molecular measurement procedures, because not all variants may have equivalent reactivity under the same reaction conditions. LoD estimates may be done separately for each significant genotype, with the largest value taken as the LoD estimate for the overall measurement procedure. Less common genotypes may be either included in the formal LoD study or checked in a subsequent verification study to ensure that their behavior is consistent with the LoD claim for the major genotypes.

5.5.1 Experimental Design

5.5.1.1 Protocol Requirements

The experimental design is used to process replicates of dilutions made from multiple independent samples of known measurand concentrations across multiple days. Some measurement procedures may not have the throughput to test the minimum number of samples and/or replicates in three days. In these cases, increasing the number of testing days to accommodate the capability of the instrument is acceptable. This study is repeated for multiple reagent lots, with an LoD calculated for each individual reagent lot if two or three reagent lots were used, or on the combined dataset from all lots if four or more

reagent lots were used. The maximum observed LoD is taken as the reported value for the measurement procedure. The LoB is either (1) defined as zero and confirmed through testing of multiple negative patient samples, or (2) determined using the classical approach with multiple negative patient samples.

The minimal experimental design is:

- Two reagent lots
- One instrument system
- Three days
- Three samples of known measurand content (positive samples)
- 30 individual negative patient samples
- Five dilutions per positive sample
- 20 replicates per dilution (across all testing days) per positive sample per reagent lot
- Two replicates (across all testing days) per negative sample per reagent lot

The dilution series should be made so that at least three dilutions yield hit rates within the range of 0.10 to 0.90 and at least one dilution yields a hit rate exceeding 0.95. It is not desirable to have more than one dilution at either extreme (ie, < 0.10 and/or > 0.95), as this could unduly impact the quality of the probit model fit and resulting LoD estimate. This may require preliminary testing with a single sample to judge the dilution ratios and the number of dilutions to be made. One useful approach is to target dilutions as a geometric series about an assumed LoD level. It is assumed that the diluent used to prepare the dilution series has been shown to provide acceptable linearity over the measurand concentration range tested and that such dilutions are commutable with native patient samples. Increasing the number of dilutions beyond the required five minimum may be useful to improve the quality of the model (eg, eight dilutions with five having hit rates falling in the range of 0.10–0.90 and three with hit rates falling in the range of 0.80–0.99).

The above text describes the minimal experimental design to yield estimates of the LoBs and LoDs. A developer may wish to expand this design to add more levels of each experimental factor listed above and/or add more design factors, depending on the nature of the measurement procedure and the desired rigor of the resulting detection capability estimates. In addition, some measurement procedures/instrumentation may not have the throughput to test the minimum number of samples/replicates in three days. In these cases, increasing the number of testing days to accommodate the capability of the instrument is acceptable. If the probit model does not provide an acceptable fit to the data, to improve the fit, it may be useful to augment the data by testing additional dilutions and increasing the number of replicates at a concentration level at which the hit rate appears to be an outlier. Consultation with a statistician may be helpful to suggest experimental design options to improve the quality of fit.

5.5.1.2 Sample Selection

Follow the guidance in Section 4.5 regarding identification and selection of appropriate negative and low level samples. These will be referred to as “starting samples.” It is acceptable to use pools of negative samples rather than all individual patient samples, but at least 30 unique negative samples must be tested.

At least three independent positive patient samples should be used. World Health Organization (WHO) standards or their equivalent, if available for the measurement procedure in question, should be included as additional positive samples. If the measurand exhibits genetic variation, sufficient samples should be selected to represent the major genotypes—particularly those specifically cited in the measurement procedure’s instructions for use (IFU). Less common or less clinically relevant genotypes may be accessed through verification testing (see Section 7.3) rather than being part of the primary evaluation testing protocol.

The genotypes to be tested and the number of samples per genotype may depend upon the measurand and/or particular device under study. Consultation with the appropriate regulatory bodies may be useful to resolve these issues before performing the evaluation study.

Negative patient samples are used to assess the false-positive rate of the measurement procedure and/or to evaluate an LoB. Native patient samples should be used for this purpose, rather than processed or artificial samples.

5.5.2 Experimental Steps

Decide on the experimental design factors and number of levels for each factor to be used (see minimal requirements in Section 5.5.1.1), as well as the processing plan to test the factors with the specific measurement procedure. Testing for the LoB and LoD may be done in parallel or sequentially as the two are handled as independent studies by this experimental approach.

5.5.2.1 Limit of Blank

Depending on the nature of the particular measurement procedure, the developer may choose to either set the LoB as zero by default, or calculate the LoB using a version of the classical approach described in Section 5.3. If the zero default option is used, then a set of negative patient samples are run as confirmatory tests to assess the false-positive rate.

Assign LoB = Zero and Confirm

1. Decide on the experimental design factors and number of levels for each factor to be used (see minimal requirements in Section 5.5.1.1), as well as the processing plan to test the factors with the specific measurement procedure.

2. Identify and prepare sufficient aliquots of all negative samples to complete the planned testing. Ensure that extra aliquots are provided to accommodate possible testing errors or processing upsets.

3. Each testing day, process the designated number of replicate tests for each sample according to the processing plan.

4. Review the measurement results each testing day to check for possible processing errors or missing results. Identify potential outliers and assignable causes for them. Outliers arising from such assignable causes—aside from analytical errors of the measurement procedure itself—may be retested and substituted into the data, ideally on the same testing day. Any such retests must be documented, along with the original test results. Presence of more than five such outliers with no identified assignable causes across all negative sample results for any one reagent lot is reason to reject and repeat the study for that reagent lot.

5. Ensure that sufficient measurement results are available at the end of testing to start data analysis (a minimum of 30 total blank negative sample results for each reagent lot).

Evaluate by Classical Approach

In case the confirmation of LoB = zero fails, follow the guidance in Section 5.3 to obtain an LoB estimate for the measurement procedure. It is acceptable to use a single instrument system in the experimental design.

5.5.2.2 Limit of Detection

1. For measurands having multiple genotypes: review all genotypes for the measurand. Identify and document which will be incorporated into the LoD testing and which, if any, will be assessed through subsequent verification testing.

2. Identify sources and obtain sufficient volumes for all samples to be used per the experimental design and processing plan for the specific measurement procedure under evaluation. At least one sample must be used per genotype to be included in the LoD testing, per Step 1 above. These will be referred to as "starting samples" in subsequent steps.

3. Prepare a series of at least five dilutions for each starting sample, per guidance in Section 5.5.1.1.

4. Each testing day, process the designated number of replicate tests per dilution according to the processing plan.

5. Review the measurement results each testing day to check for possible processing errors or missing results. Identify potential outliers and assignable causes for them. Outliers arising from such assignable causes—aside from analytical errors of the measurement procedure itself—may be retested and substituted into the data, ideally on the same testing day. Any such retests must be documented, along with the original test results.

6. Repeat Steps 3 to 5 for each starting sample and across all reagent lot/instrument system combinations, per the experimental design and processing plan.

7. Ensure that sufficient measurement results are available at the end of testing to start data analysis (a minimum of 20 replicates per dilution for each reagent lot).

5.5.3 Data Analysis

The analysis process uses the following sequence of steps to provide the final LoB and LoD estimates:

1. Select the α error risk to use for the LoB (either calculating the estimate per the protocols of Section 5.3 or confirming the default zero value) and the β error risk to use for the LoD (typically $\alpha = \beta = 0.05$).

2. Define or calculate the LoB based on the approach selected for the measurement procedure.

3. Calculate the LoD for each reagent lot.

4. Select the maximum LoD observed as the LoD estimate for the measurement procedure.

5.5.3.1 Calculation of Limit of Blank

Assign LoB = Zero, and Confirm

1. Set LoB = zero for the measurement procedure.

2. For each reagent lot separately, count the number of negative sample replicate measurement results across all samples, instrument lots, etc., that would be reported as a positive result. Convert this value into a percentage with respect to the number of all negative sample replicates processed, and report as the percentage of false-positive results.

3. If the percentage of false-positive results for a given reagent lot does not exceed 100α%, then the 100(1 − α)% of the result is zero and LoB = zero is confirmed for that lot. Each reagent lot must be confirmed separately.

Evaluate by Classical Approach

Follow the protocol described in Section 5.3 to obtain the LoB estimate for the measurement procedure.

5.5.3.2 Calculation of Limit of Detection

Analyze the data from all starting samples for each reagent lot independently.

1. Calculate the hit rate H_i for each dilution as:

$$H_i = \frac{Npos_i}{Ntot_i} \quad (12)$$

where $Npos_i$ is the number of replicates reported as positive for presence of the measurand and $Ntot_i$ is the total number of replicates processed for the *i*th dilution.

2. Using computer software for a direct probit analysis, input the hit rates (y-axis variable) with their corresponding measurand concentrations (x-axis variable), then perform a probit fit. Using log10 concentration on the x-axis often improves the probit fit.

3. Evaluate the quality of the probit model fit to the data by a suitable statistical test (eg, comparing the deviance statistic or Pearson chi-square statistic to the quantile of chi-square distribution). If the goodness of fit is not acceptable, it may be possible to improve the goodness of fit by testing additional dilutions and/or replicates at the current dilutions, as appropriate, and combining these results with the existing data.

4. If the model fit is deemed acceptable, use the software to obtain the measurand concentration corresponding to the desired β error risk (typically β = 0.05 for a hit rate of 0.95) and report this as the trial LoD for that particular lot. Continue with Step 5.

5. Follow Steps 1–4 to calculate trial LoD results for each lot.

6. Review all of the trial LoD values and select the maximum as the reported LoD estimate for the measurement procedure.

6 Protocol for Evaluation of the Limit of Quantitation

6.1 Introduction

As discussed in Section 4.1, the LoQ represents the lowest measurand concentration that can be measured with respect to predefined accuracy goals. It is a characteristic of the measurement procedure with respect to both the particular performance attributes used to define the accuracy goals as well as the values for those that are deemed to be acceptable. The more stringent the acceptance requirements, the larger the LoQ is likely to be. Given the flexibility of the LoQ definition, it is necessary to include the underlying accuracy goals when reporting an LoQ estimate. The LoQ, by definition, is a performance attribute applicable only to quantitative measurement procedures.

This section describes a protocol to estimate the LoQ of a measurement procedure. It is based upon the minimal design requirements for an LoD evaluation by the classical approach (see Section 5.3.1). This is simply one design out of many that could be used to estimate the LoQ. Whichever design is selected, it should be appropriate for the measurement procedure and its applications, technically and statistically sound, and consistent with both the desired accuracy goals and the minimal design requirements listed in Section 6.3.

The LoQ may be evaluated during development of a measurement procedure. Laboratories also may wish to establish their own LoQs, possibly using different accuracy goals than were chosen originally by the developer. Depending on the acceptance goals, the LoQ could be equal to or greater—but never lower—than the LoD. It may not be necessary to determine the LoQ if the measurement uncertainty (or TE) can be determined and reported for all results at low-measurand concentrations, allowing the user to interpret whether or not it is suitable for use.

The protocol is described in terms of underlying experimental design, sample requirements, procedural steps, and data analysis. It should be understood that the protocol description is based on the minimally acceptable experimental design requirements. Depending on the particular measurement procedure and desired statistical rigor of the resulting estimates, it may be appropriate to augment the number of factors in the experimental design, the number of levels of some factors, and/or the number of replicate measurements to be acquired.

6.2 Specification of Accuracy Goals

The LoQ is defined preferentially in terms of a TE goal or with respect to goals for both bias and precision. This is equivalent to the concept of "target uncertainty" in VIM[9] and CLSI document C51.[27] There may be situations, however, where bias cannot be determined at the appropriate measurand level and within-laboratory precision is used as the sole acceptance goal. In such cases, the LoQ would be equivalent to the older—and now deprecated—term functional sensitivity (see Section 4.2). Other measurement procedure performance attributes may be incorporated in an LoQ definition as appropriate for a given measurement procedure and its intended use.

No single definition of LoQ is suitable for all measurement procedures and their applications. Two of the more widely accepted definitions are TE calculations: the classical Westgard model[28] and the root mean square (RMS) or variance model.[29] These are based upon a combination of bias and precision estimates for a measurement procedure, evaluated at a specified measurand concentration. Other LoQ definitions may be used as appropriate.[30]

Westgard model $$TE = |Bias| + 2s \quad (13)$$

RMS model $$TE = \sqrt{s^2 + Bias^2} \quad (14)$$

In addition to differences among LoQ definitions, studies reported in the literature, as well as claims cited in the IFUs for different IVD products, have used different approaches in defining and calculating the underlying bias and precision terms. Bias has been defined with respect to a reference or a comparison measurement procedure, as well as recovery of a standard with an assigned value. Similarly, precision has been estimated in different studies by simple repeatability, within-laboratory precision from a long-term (see CLSI document EP05[22]) or short-term (see CLSI document EP15[23]) study, and long-term precision incorporating multiple reagent lots and calibration cycles, among other approaches.

In general, bias often is best estimated through recovery of standards or samples having an accepted reference value. Consensus-assigned standards are desirable for such testing but are not available for all measurands and are rarely at concentrations appropriate for LoQ evaluations. If consensus standards are

not available, other suitable starting materials may be patient samples or spiked solutions whose measurand concentrations are assigned by or traceable to a reference measurement procedure or other measurement procedure of acceptable accuracy. Dilutions of such materials may be used, assuming that the diluent has been shown to be compatible with the measurement procedure under evaluation and demonstrates linearity into the low concentration region.

Estimation of bias through measurement procedure comparison experiments is not generally desirable, because such studies encompass a wide range of measurand concentrations rather than focusing just at the low end of the measuring interval. There is potential impact from scatter about the regression line, change in variability of the measurement procedure with increasing measurand concentration, and influence of elevated samples far removed from the LoQ region of interest.

Precision estimates for LoQ application should reflect both repeatability and day-to-day variability. It may be appropriate to include additional sources of variability such as operator-to-operator, calibration cycle-to-cycle, etc., depending on the measurement procedure and its applications. Repeatability alone is not a good basis for an LoQ study.

Once the LoQ definition is selected, it is necessary to provide its associated accuracy goal value. As with the definition itself, no single type or source of accuracy goals would be suitable for all measurement procedures and applications. Examples of such sources that have been used include those based upon clinical utility (eg, 10% within-laboratory precision—no associated bias goal—for cardiac troponin[31]), TE-based quality goals (eg, Clinical Laboratory Improvement Amendment requirements for analytical quality,[32] Guidelines of the German Federal Medical Council [RiliBÄK] quality guidelines[33]), and quality goals based upon biological variation.[34]

6.3 Experimental Design

The design presented here is based upon the minimum design requirements for evaluation of LoD using the classical approach (see Section 5.3.1). A target concentration is selected as a trial LoQ and multiple low level samples are prepared at that concentration. The samples are processed in replicate with multiple reagent lots, using one or more instrument systems over multiple days. The design uses the Westgard TE model as its LoQ definition (other definitions may be used as appropriate). TE is calculated for each reagent lot from the measurement results. If the TE for each reagent lot meets the predefined goals, the mean concentration is reported as the LoQ for the measurement procedure.

The minimal experimental design is:

- Two reagent lots
- One instrument system
- Three days
- Three replicates per sample (for each reagent lot, instrument system, and day combination)
- Four independent low level samples of known measurand concentration
- 36 total low level sample replicates per reagent lot (across all low level samples, instrument systems, and days)

The developer may wish to add more factors and/or to increase the number of replicates beyond the minimum in order to increase the rigor of the resulting LoQ estimates.

The only significant difference between this design and the classical approach LoD design is in selection of the low level samples. A sample concentration interval is used for the LoD design, while a single target concentration is used for the LoQ design.

Measurements should be acquired from multiple, independent low level samples or pools of samples in order to account for matrix variability among samples. At least four samples should be used in the study. To the extent possible, these should be commutable with native patient samples.

An assigned value must be known for each of the samples if the LoQ definition will incorporate a bias component. This may be from a previous external assignment (such as for WHO and similar reference standards) or assigned by a reference measurement procedure—or other measurement procedure of acceptable accuracy—with adjustment for dilution as appropriate, ideally from certified reference materials having assigned values with appropriate metrological traceability.

6.4 Experimental Steps

1. Decide on the experimental design factors and number of levels for each factor to be used, as well as the processing plan to test the factors with the specific measurement procedure.

2. Decide on the LoQ definition and associated accuracy goals for the measurement procedure, as well as the trial LoQ measurand level.

3. Obtain low level samples targeted at the trial LoQ. Each sample should have an associated known value (R_i), obtained from a reference measurement procedure, theoretical spike or dilution calculation from a known starting measurand concentration, or other similar source, to allow estimation of bias. Prepare sufficient aliquots of them to complete the planned testing. Ensure that extra aliquots are provided to accommodate possible testing errors or processing upsets.

4. Each testing day, process the designated number of replicate tests for each sample according to the processing plan.

5. Review the measurement results each testing day to check for possible processing errors or missing results. Identify potential outliers and assignable causes for them. Outliers arising from such assignable causes—aside from analytical errors of the measurement procedure itself—may be retested and substituted into the data, ideally on the same testing day. Any such retests must be documented, along with the original test results. Presence of more than five such outliers identified for assignable causes across all low level sample results from any one reagent lot is reason to reject and repeat the study for that reagent lot.

6. Ensure that sufficient measurement results are available at the end of testing to start data analysis. A minimum of 36 total low level sample results at the trial LoQ measurand level are required per reagent lot.

6.5 Data Analysis

The data analysis process uses the following sequence of steps to provide the final LoQ estimate. The Westgard TE model is used here as the LoQ definition to illustrate the calculations. The actual calculation steps will depend upon the LoQ definition selected for a particular measurement procedure; however, the basic approach to data analysis will be the same.

Analyze the data for each reagent lot independently if there are two or three lots, or use the combined dataset across all lots if four or more lots were used.

1. Calculate the average value ($\bar{x}$) and SD(s) for each low level sample across all replicates for the given reagent lot.

2. Calculate the bias for each low level sample from its assigned value (R).

$$Bias = \bar{x} - R \quad (15)$$

3. Calculate the TE for each sample using the Westgard model, per equation 13. Convert to %TE units if needed, with respect to the sample's assigned value.

4. Repeat Steps 1 to 3 to calculate the observed TE for each sample for all other reagent lots, if two or three lots were used.

5. Review the observed TE estimates for each reagent lot against the predefined accuracy goal. For each reagent lot, the sample with the lowest concentration that met the accuracy specifications is taken as the LoQ for the lot.

6. The greatest LoQ across all lots (if two or three lots were used) or the LoQ from the combined dataset (if four or more reagent lots were used) is taken as the LoQ for the measurement procedure.

If all of the samples for one or more of the reagent lots fail to meet the accuracy goal, the entire study needs to be repeated with a new set of low level samples targeted to a greater measurand concentration.

Worked examples are given in Appendix D for establishment of LoQ claims.

6.6 Variant Approach: Combined Limits of Detection and Quantitation Evaluation

The above experimental design and data analysis are based on *a priori* selection of a single measurand concentration for testing. Depending on the measurement procedure and its associated accuracy goals, a variant approach may be appropriate that enables evaluation of the LoQ as part of an LoD evaluation using the precision profile approach (see Section 5.4). The only significant change in the testing procedure is that the low level samples must have known measurand concentrations to allow calculation of bias—if that is required for the particular accuracy goal to be used.

With suitable samples in hand, follow the experimental design and steps, per Sections 5.4.1–5.4.2. Follow Steps 1 to 3 in Section 6.5 to calculate TE estimates (or other estimate as required for the particular accuracy goal) for each sample for each reagent lot. Plot the observed TEs (y-axis) vs the sample measurand concentrations (x-axis) to give a TE profile, and fit by a suitable regression model or graphical interpolation. Using the plot or regression model, determine the measurand concentration that corresponds to the LoQ accuracy goal. Report this as the LoQ for the measurement procedure.

This combined LoD/LoQ evaluation may be successful because of the relatively wide range of measurand concentrations that typically go into the precision profile approach. This is particularly useful if the LoQ is determined solely on the basis of a precision requirement. A combined evaluation based on the classical LoD approach (see Section 5.3) also might be viable if the LoQ accuracy goals are broad enough such that the LoQ would be expected to lie in close proximity to the LoD.

7 Verification of Detection Capability Claims

7.1 Introduction

Verification testing is used to ensure that performance of a measurement procedure in standard practice is consistent with claims established by the developer. For detection capability, the LoB and LoD would be routinely verified, as well as the LoQ, if it is defined for the measurement procedure.

A common verification approach is used for each of the detection capability claims. A small number of samples are tested in replicate over multiple days using a single reagent lot and a single instrument system. The proportion of measurement results that are consistent with the respective claim is calculated and compared to the appropriate boundary value, shown in Table 1 ($\alpha = 0.05$ or $\beta = 0.05$), in order to determine the outcome of the verification. If the observed proportion is less than the value provided in Table 1, one may conclude that the observed results are not consistent with the claim.

Consider an example LoB verification study performed using $N = 20$ measurements in which 17 results fell below the LoB claim. For this N, the proportion is 85% (17/20) with an associated upper one-sided 95% confidence limit of 93.8%. This upper confidence limit value falls short of the definition of LoB as the highest blank measurement that can be observed with a stated (ie, 95%) probability. Therefore, the verification outcome would be "fail." If, however, 18 out of the 20 measurements fell below the LoB claim, the proportion would become 90% (18/20) with an associated upper one-sided 95% confidence level of 96.6%. This upper confidence limit value exceeds the LoB definition and the verification outcome would be "pass."

NOTE: The underlying hypothesis testing uses H_0: $\mu \geq 95\%$ vs H_0: $\mu < 95\%$. Calculation of the 95% confidence intervals was done using a score method as described in CLSI document EP12.[36]

Table 1. Bounds for Observed Proportion of Results Relative to a Detection Capability Claim
(modified from Linnet and Kondratovich[20])

Total Number of Measurements in Study (N)	Observed Proportion Boundary
20	85%
30	87%
40	88%
50	88%
60	90%
70	90%
80	90%
90	91%
100	91%
150	92%
200	92%
250	92%
300	93%
400	93%
500	93%
1000	94%

The verification protocols below are described in terms of experimental design, sample requirements, procedural steps, and data analysis. It should be understood that these are based on minimally acceptable experimental design requirements. Depending on the particular measurement procedure and desired statistical rigor of the resulting estimates, it may be appropriate to augment the number of factors in the

experimental design, the number of levels of some factors, and/or the number of replicate measurements to be acquired.

Worked examples of verification testing are provided in Appendixes E and F.

In addition to formal verification testing, additional insight into detection capability of measurement procedures may be gained from examination of results from proficiency testing programs that distribute materials containing very low measurand levels.[35]

7.2 Verification of a Limit of Blank Claim

7.2.1 Protocol Requirements

The minimal experimental design is:

- One reagent lot
- One instrument system
- Three days
- Two blank samples
- Two replicates per sample per day
- 20 total blank replicates (across all samples and days)

The minimal design described above (ie, one instrument system, three days, two samples, two replicates) does not yield the necessary 20 total replicates per reagent lot. It is necessary for the developer to increase one or more design factors to provide a sufficient number of measurement results. The selection of which factors to increase depends on the particular measurement procedure and available resources for testing. The developer also may wish to add more factors and/or to increase the number of replicates beyond the minimum in order to increase the power of the verification experiment. Follow the guidance in Section 4.5 regarding identification and selection of appropriate blank samples.

7.2.2 Experimental Steps and Data Analysis

1. Decide on the experimental design factors and number of levels for each factor to be used, as well as the processing plan to test the factors with the specific measurement procedure.

2. Prepare sufficient aliquots of all blank samples to complete the planned testing. Ensure that extra aliquots are provided to accommodate possible testing errors or processing upsets.

3. Each testing day, process the designated number of replicate tests for each sample according to the processing plan.

4. Review the measurement results each testing day to check for possible processing errors or missing results. Identify potential outliers and assignable causes for them. Outliers arising from such assignable causes—aside from analytical errors of the measurement procedure itself—may be retested and substituted into the data, ideally on the same testing day. Any such retests must be documented along with the original test results. Presence of more than two such outliers identified for assignable causes across all blank sample results is reason to reject and repeat the study.

5. Ensure that sufficient measurement results are available at the end of testing to start data analysis. A minimum of 20 total blank sample results are required.

6. Calculate the percentage of all blank measurement results that are less than or equal to the LoB claim.

7. Compare the percentage from Step 6 to the lower bound value in Table 1 for the total number of blank measurement results in the verification study (N). Use the row nearest to N if there is no exact match.

8. If the observed percentage is greater than or equal to the Table 1 value, the verification is deemed to be successful and the claim is taken as verified.

9. If the observed percentage is less than the Table 1 value, the verification was not successful. Review the measurement results for possible errors and perform troubleshooting as appropriate to look for potential causes for the failure. Contact the measurement procedure developer for assistance, if needed. Depending on the outcome, either perform a new verification study or establish an LoB claim using an evaluation protocol, per Section 5.

7.3 Verification of a Limit of Detection Claim

7.3.1 Protocol Requirements

The minimal experimental design is:

- One reagent lot
- One instrument system
- Three days
- Two samples at the LoD claim measurand concentration
- Two replicates per sample per day
- 20 total low level replicates (across all samples and days)

The minimal design described above (ie, one instrument system, three days, two samples, two replicates) does not yield the necessary 20 total replicates per reagent lot. It is necessary for the developer to increase one or more design factors to provide a sufficient number of measurement results. The selection of which factors to increase depends on the particular measurement procedure and available resources for testing. The developer also may wish to add more factors and/or to increase the number of replicates beyond the minimum in order to increase the power of the verification experiment. Follow guidance in Sections 4.5 and 5.3.1.2 for criteria on selection of suitable low level samples.

It is necessary to have the associated LoB claim in order to verify an LoD claim. The initial step of the following experimental procedure is to either verify or establish an LoB claim.

7.3.2 Experimental Steps and Data Analysis

1. If an LoB claim is provided, follow the protocol in Section 7.2.1 to verify it. If the verification is successful, use that LoB claim. If the verification is not successful, or if an LoB claim is not given, then establish an LoB claim using an evaluation protocol, per Section 5.

2. Decide on the LoD experimental design factors and number of levels for each factor to be used, as well as the processing plan to test the factors with the specific measurement procedure.

3. Prepare sufficient aliquots of all low level samples to complete the planned testing. Ensure that extra aliquots are provided to accommodate possible testing errors or processing upsets.

4. Each testing day, process the designated number of replicate tests for each sample according to the processing plan.

5. Review the measurement results each testing day to check for possible processing errors or missing results. Identify potential outliers and assignable causes for them. Outliers arising from such assignable causes—aside from analytical errors of the measurement procedure itself—may be retested and substituted into the data, ideally on the same testing day. Any such retests must be documented, along with the original test results. Presence of more than two such outliers identified with no assignable causes across all low level sample results is reason to reject and repeat the study.

6. Ensure that a sufficient number of measurement results are available at the end of testing to start data analysis. A minimum of 20 total low level sample results are required.

7. Calculate the percentage of all low level measurement results that are equal to or exceed the LoB claim.

8. Compare the percentage from Step 7 to the lower bound value in Table 1 for the total number (N) of measurement results from low level samples in the verification study. Use the row nearest to N if there is no exact match.

9. If the observed percentage is greater than or equal to the Table 1 value, the verification is deemed to be successful and the claim is taken as verified.

10. If the observed percentage is less than the Table 1 value, the verification was not successful. Review the measurement results for possible errors and perform troubleshooting as appropriate to look for potential causes for the failure. Contact the measurement procedure developer for assistance if needed. Depending on the outcome, either perform a new verification study or establish an LoD claim using an evaluation protocol, per Section 5.

7.4 Verification of a Limit of Quantitation Claim

The following protocol is suitable for verification of LoQ claims using a TE-based accuracy goal. LoQ claims that are based solely upon a precision goal may be verified with the precision experiment outlined in CLSI document EP15.[23]

7.4.1 Protocol Requirements

The minimal experimental design is:

- One reagent lot
- One instrument system
- Three days
- Two samples at the LoQ claim measurand concentration
- Two replicates per sample per day
- 20 total low level replicates (across all samples and days)

The minimal design described above (ie, one instrument system, three days, two samples, two replicates) does not yield the necessary 20 total replicates per reagent lot. It is necessary for the developer to increase one or more design factors to provide a sufficient number of measurement results. The selection of which factors to increase depends on the particular measurement procedure and available resources for testing. The developer also may wish to add more factors and/or to increase the number of replicates beyond the minimum in order to increase the power of the verification experiment. Follow guidance in Sections 4.5 and 6.2 for criteria on selection of appropriate samples with known measurand concentration targeted to the LoQ claim.

A target value must be known for each of the samples. This may be obtained from known external assignment (eg, a reference standard) or assignment by a reference or other measurement procedure of acceptable accuracy, with adjustment for dilution, as appropriate.

7.4.2 Experimental Steps and Data Analysis

1. Decide on the experimental design factors and number of levels for each factor to be used, as well as the processing plan to test the factors with the specific measurement procedure.

2. Prepare sufficient aliquots of all samples to complete the planned testing. Ensure that extra aliquots are provided to accommodate possible testing errors or processing upsets.

3. Each testing day, process the designated number of replicate tests for each sample according to the processing plan.

4. Review the measurement results each testing day to check for possible processing errors or missing results. Identify potential outliers and assignable causes for them. Outliers arising from such assignable causes—aside from analytical errors of the measurement procedure itself—may be retested and substituted into the data, ideally on the same testing day. Any such retests must be documented, along with the original test results. Presence of more than two such outliers identified for assignable causes across all results is reason to reject and repeat the study.

5. Ensure that a sufficient number of measurement results are available at the end of testing to start data analysis. A minimum of 20 total sample results are required.

6. For each sample, calculate the allowable error window around its target value (eg, if the accuracy goal is ± 15%, the error window would be target ± 15%).

7. Count the number of results for each sample that fall within its respective allowable error window. From these, calculate the percentage of all sample measurement results that meet the acceptance goals criteria for the LoQ claim.

8. Compare the percentage from Step 7 to the lower bound value in Table 1 for the total number of measurement results in the verification study (N). Use the row nearest to N if there is no exact match.

9. If the observed percentage is greater than or equal to the Table 1 value, the verification is deemed to be successful and the claim is taken as verified.

10. If the observed percentage is less than the Table 1 value, the verification was not successful. Review the measurement results for possible errors and perform troubleshooting as appropriate to look for potential causes for the failure. Contact the measurement procedure developer for assistance if needed. Depending on the outcome, either perform a new verification study or establish an LoQ claim using an evaluation protocol per Section 6.

8 Reporting Detection Capability

The information in the following section applies for quantitative clinical laboratory measurement procedures. For information relative to qualitative measurement procedures see CLSI document EP12.[36]

8.1 Interpretations and Reporting Intervals for Quantitative Measurement Procedure Results

Measured values less than the LoQ but greater than the LoB may be used to show that the analyte is present, but the actual measured levels should not otherwise be used for clinical interpretation. Results between the LoD and LoQ could be reported, possibly with an associated cautionary notice regarding higher uncertainty in the result. There are situations in which laboratories may report measurement results regardless of whether the values are below or exceed the LoD. Examples of these include the use of the average of replicates as a subject's result, or for scientific studies.

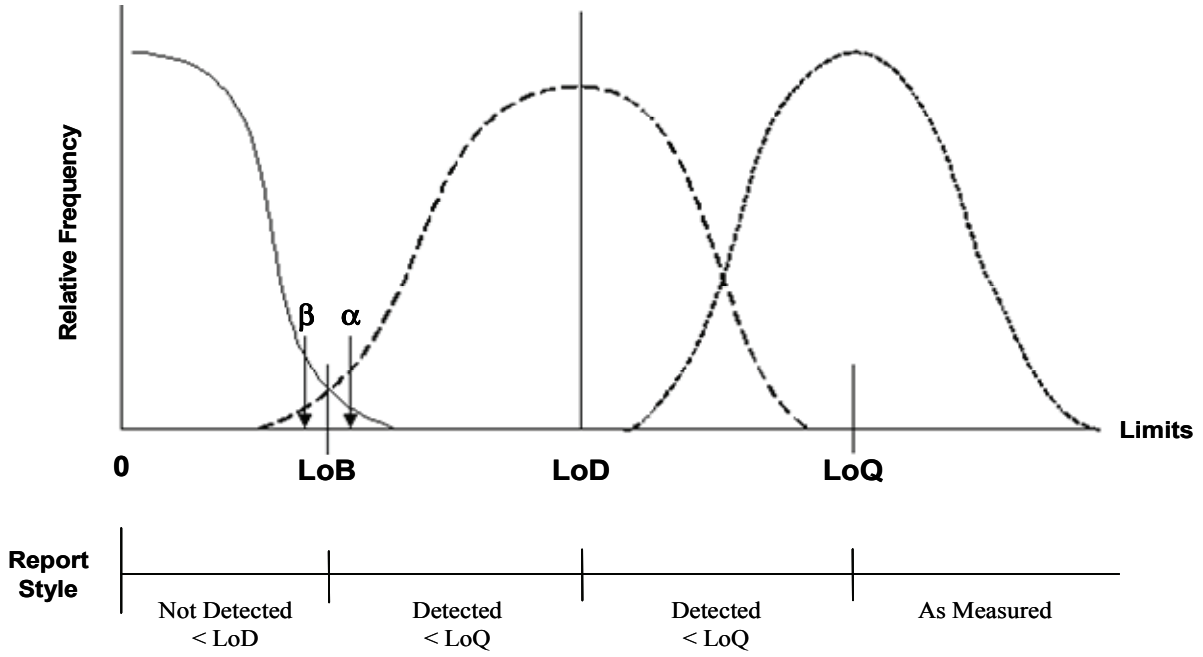
How measurement results are reported to clients depends on a laboratory's standard procedures and where the observed result lies relative to the detection capability limits of the measurement procedure. If a laboratory wishes to report the most complete information—including a "gray zone" in which quantitation is uncertain—then the following style may be appropriate:

Result ≤ LoB	Report "not detected"; result "< LoD."
LoB < Result < LoD	Report "analyte detected"; result "< LoQ."
LoD ≤ Result < LoQ	(a) Report "analyte detected"; result "< LoQ"; or (b) Report the result with a caution about possibly higher uncertainty.
Result ≥ LoQ	Report the result.

If a laboratory chooses to report only quantitative results or "less than" determinations, then the following simplified style may be considered:

Result ≤ LoB	Report "not detected."
LoB < Result < LoQ	Report "result < LoQ" or "detected."
Result ≥ LoQ	Report quantitative result.

The relationships of these reporting schemes to the detection capability limits are shown in Figure 4.



Abbreviation: LoB, limit of blank; LOD, limit of detection; LoQ, limit of quantitation.

Figure 4. Alignment of Suggested Reporting Formats With Detection Capability Limits. (Distribution of results shown for a blank sample, low level sample at LoD, and a low level sample at LoQ.)

As an example of these reporting styles, consider a measurement procedure whose LoB is 6 mmol/L, LoD is 8 mmol/L, and LoQ is 10 mmol/L. The following are options for reports that could accompany various test results.

For a laboratory reporting the most complete information:

Example Result	Report As
5 mmol/L	"Substance not detected; result < 8 mmol/L."
7 mmol/L	"Substance present, cannot be quantified; result < 10 mmol/L."
9 mmol/L	(a) "Substance present, cannot be quantified; result < 10 mmol/L"; or (b) "Result = 9 mmol/L, but should be interpreted with caution because of a higher level of uncertainty" (report uncertainty, if requested).
11 mmol/L	"Result = 11 mmol/L" (report uncertainty or goal, if requested).

For a laboratory reporting only quantitative results:

Result	Report Option 1	Report Option 2
5 mmol/L	"Not detected."	"Not detected."
7 mmol/L	"Result < 10 mmol/L."	"Detected; < 10 mmol/L."
9 mmol/L	"Result < 10 mmol/L."	"Detected; < 10 mmol/L."
11 mmol/L	"Result = 11 mmol/L."	"11 mmol/L."

8.2 Example Labeling for Detection Capability Claims of Quantitative Measurement Procedures

Reports of LoD claims or studies should include at least the following information:

- Measurand name
- LoD estimate
- α and β error risks
- Associated LoB estimate
- Total number of determinations across all blank samples
- Total number of determinations across all positive samples

The following is an example of wording for an LoD claim in a product IFU:

> "The LoD for C-reactive protein is 3 mg/L, determined consistent with the guidelines in CLSI document EP17 and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 130 determinations, with 70 blank and 60 low level replicates; and an LoB of 1 mg/L."

Reports of LoQ claims or studies should include at least the following information:

- Measurand name
- LoQ estimate
- Definition of the accuracy goal(s)
- Bias and/or precision components, if they are part of the LoQ definition (eg, TE)
- Total number of determinations across all samples

The following is an example of wording for an LoQ claim in a product IFU:

> "The LoQ for C-reactive protein is 7 mg/L, determined consistent with the guidelines in CLSI document EP17, based on 130 determinations; and a TE goal of 13.5% calculated using the RMS error model.[a] The associated bias and precision components were 4.6 mg/L and 5.4 mg/L, respectively."

Another option would be to state an LoQ claim in terms of target uncertainty, such as:

> "The LoQ for C-reactive protein is 7 mg/L, determined consistent with the guidelines in CLSI document EP17, based on 130 determinations; and a target expanded uncertainty goal of 13.5%, based on 95% coverage and a coverage factor of k = 2."

[a] Macdonald R. Quality assessment of quantitative analytical results in laboratory medicine by root mean square of measurement deviation. *J Lab Med.* 2006;30(3):111-117.

References

[1] ISO. *Capability of detection – Part 1: Terms and definitions.* ISO 11843-1. Geneva, Switzerland: International Organization for Standardization; 1997.

[2] ISO. *Capability of detection – Part 2: Methodology in the linear calibration case.* ISO 11843-2. Geneva, Switzerland: International Organization for Standardization; 2000.

[3] ISO. *Capability of detection – Part 3: Methodology of determination of the critical value for the response variable when no calibration data are used.* ISO 11843-3. Geneva, Switzerland: International Organization for Standardization; 2003.

[4] ISO. *Capability of detection – Part 4: Methodology for comparing the minimum detectable value with a given value.* ISO 11843-4. Geneva, Switzerland: International Organization for Standardization; 2003.

[5] ISO. *Capability of detection – Part 5: Methodology in the linear and non-linear calibration cases.* ISO 11843-5. Geneva, Switzerland: International Organization for Standardization; 2008.

[6] Miller MJ, Astles JR, Baszler T, et al.; National Center for Emerging and Zoonotic Infectious Diseases, CDC. Guidelines for safe work practices in human and animal medical diagnostic laboratories. *MMWR Surveill Summ.* 2012;61:1-102.

[7] CLSI. *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition.* CLSI document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.

[8] ISO. *Statistics – Vocabulary and symbols – Part 1: Probability and general statistical terms.* ISO 3534-1. Geneva, Switzerland: International Organization for Standardization; 1993.

[9] Bureau International des Poids et Mesures (BIPM). *International Vocabulary of Metrology – Basic and General Concepts and Associated Terms* (VIM, 3rd edition, JCGM 200:2012). http://www.bipm.org/en/publications/guides/vim.html. Accessed May 14, 2012.

[10] ISO. *In vitro diagnostic medical devices – Information supplied by the manufacturer (labelling) – Part 1: Terms, definitions, and general requirements.* ISO 18113-1. Geneva, Switzerland: International Organization for Standardization; 2009.

[11] CLSI/NCCLS. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.* CLSI/NCCLS document EP06-A. Wayne, PA: NCCLS; 2003.

[12] Currie LA. Limits for qualitative detection and quantitative determination. *Anal Chem.* 1968;40(3):586-593.

[13] Adams PB, Passmore WO, Campbell DE. Symposium on trace characterization – chemical and physical; paper no. 14. Washington, DC: National Bureau of Standards; 1966.

[14] Spencer CA, Takeuchi, M, Kazarosyan. Current status and performance goals for serum thyrotropin (TSH) assays. *Clin Chem.* 1996;42(1):140-145.

[15] Currie LA. Nomenclature in evaluation of analytical methods, including detection and quantification capabilities. *Pure Appl Chem.* 1995; 67(10):1699-1723.

[16] CLSI. *Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline—Second Edition.* CLSI document MM03-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2006.

[17] CLSI. *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition.* CLSI document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.

[18] CLSI. *Evaluation of Matrix Effects; Approved Guideline—Second Edition.* CLSI document EP14-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.

[19] ISO. *Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.* ISO 5725-2. Geneva, Switzerland: International Organization for Standardization; 1994.

[20] Linnet K, Kondratovich M. Partly nonparametric approach for determining the limit of detection. *Clin Chem.* 2004;50(4):732-740.

[21] Ekins RP. The "precision profile": its use in RIA assessment and design. *Ligand Quarterly.* 1981;4:33-44.

[22] CLSI/NCCLS. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.* CLSI/NCCLS document EP05-A2. Wayne, PA: NCCLS; 2004.

[23] CLSI. *User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition.* CLSI document EP15-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2006.

[24] Sadler WA, Smith MH, Leggs HM. A method for direct estimation of imprecision profiles, with reference to immunoassay data. *Clin Chem.* 1988;34(6):1058-1061.

[25] Bliss CI. The method of probits. *Science.* 1934; 79(2037):38-39.

[26] Burd EM. Validation of laboratory-developed molecular assays for infectious diseases. *Clin Microbiol Rev.* 2010;23(3):550-576.

[27] CLSI. *Expression of Measurement Uncertainty in Laboratory Medicine; Approved Guideline.* CLSI document C51-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

[28] Westgard JO, Carey RN, Wold S. Criteria for judging precision and accuracy in method development and evaluation. *Clin Chem.* 1974;20(7):825-833.

[29] Macdonald R. Quality assessment of quantitative analytical results in laboratory medicine by root mean square of measurement deviation. *J Lab Med.* 2006;30(3):111-117.

[30] Petersen PH, Stöckl D, Westgard JO, Sandberg S, Linnet K, Thienpont L. Models for combining random and systematic errors: assumptions and consequences for different models. *Clin Chem Lab Med.* 2001;39(7):589-595.

[31] Wu AHB, Apple FS, Gibler WB, Jesse RL, Warshaw MM, Valdes R Jr. National Academy of Clinical Biochemistry Standards of Laboratory Practice: recommendations for the use of cardiac markers in coronary artery diseases. *Clin Chem.* 1999;45(7):1104-1121.

[32] 57 Federal Register 7002. *Medicare, Medicaid and CLIA Programs; Regulations Implementing the Clinical Laboratory Improvement Amendments of 1988; Final Rule.* (Codified at 42 CFR Part 405 et al.); 1992.

[33] Richtlinie der Bundesärztekammer zur Qualitätssicherung Laboratoriumsmedizinischer Untersuchungen. *Deutsches Ärzteblatt.* 2008;105(7):A341-A355. http://www.bundesaerztekammer.de/downloads/rililabor2008koor.pdf. Accessed May 14, 2012.

[34] Antonsen S, Petersen PH, Fraser CG. Medical need for quality specifications in clinical laboratories: quality specifications for detection limit. *Ups J Med Sci.* 1993;98(3):317-321.

[35] Beckett G, MacKenzie F. Thyroid guidelines - are thyroid-stimulating hormone assays fit for purpose? *Ann Clin Biochem.* 2007;44 (Pt 3):203-208.

[36] CLSI. *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition.* CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

Appendix A. Worked Example: Evaluation of Limits of Blank and Detection by the Classical Approach

Data for this example came from a detection capability study for an estradiol measurement procedure. Because initial precision testing results data showed repeatability to be relatively consistent for samples at the low end of the measuring interval, the classical approach protocol was used to evaluate the limit of blank (LoB) and limit of detection (LoD).

The experimental design listed below was selected to meet the minimum requirements in Section 5.3.1.1 of this document. The default values of $\alpha = \beta = 0.05$ were used for the Type I and II error risks.

- Two reagent lots (one and two)
- One instrument system
- Three test days
- Five blank samples
- Five low level samples
- Four replicate measurements per sample for each reagent-day combination
- 60 each total blank and low level sample measurements per reagent lot
  (3 days × 5 samples × 4 replicates)

Blank samples were made by immunoadsorption of individual patient serum samples to remove endogenous estradiol. A batch mode repeatability study (n = 20) was performed using the zero level calibrator as the sample. The maximum observed measurement result was 7 pg/mL. This was used as an initial LoB estimate and to identify the desired range for selecting low level samples as 7–35 pg/mL (one to five times the estimated LoB, per Section 5.3.1.2 of this document). Five patient samples were identified that fell into this range from a separate method comparison study.

The study was run per the above design. Measurements were acquired as instrument signal responses and converted to analyte values through offline calibration to avoid censoring of data for the blank samples. Tables A1 through A4 list the observed blank and low level sample results across the two reagent lots.

Table A1. Observed Blank Sample Results for Reagent Lot 1 (Units are pg/mL)

Day	Replicate	Blank 1	Blank 2	Blank 3	Blank 4	Blank 5
1	1	2.6	1.0	−4.4	1.5	1.2
	2	−0.8	2.9	−3.4	−1.9	−0.7
	3	5.5	4.9	7.0	5.1	6.1
	4	6.0	8.0	6.9	5.7	5.1
2	1	4.5	6.9	4.3	4.1	4.8
	2	0.6	5.0	3.2	4.5	3.3
	3	−2.3	3.4	−1.4	−0.6	−2.8
	4	3.4	1.2	−4.2	0.5	−1.4
3	1	5.9	6.5	5.9	5.4	8.7
	2	7.6	5.6	7.6	7.6	3.6
	3	4.1	−2.2	3.8	4.4	5.1
	4	−1.4	2.3	5.8	6.6	3.5

Appendix A. (Continued)

Table A2. Observed Low Level Sample Results for Reagent Lot 1 (Units are pg/mL)

Day	Replicate	Low 1	Low 2	Low 3	Low 4	Low 5
1	1	21.0	13.3	12.8	17.3	19.2
	2	22.8	12.6	12.9	19.2	22.7
	3	28.2	18.2	17.4	21.5	28.3
	4	25.9	14.7	16.0	22.2	26.2
2	1	26.4	17.8	15.9	24.1	25.1
	2	28.3	14.0	14.1	25.8	30.3
	3	20.7	14.1	11.3	16.0	23.4
	4	21.9	12.5	9.4	16.4	19.2
3	1	24.7	11.3	10.6	24.9	26.3
	2	22.5	12.2	13.6	23.8	23.1
	3	28.5	16.2	17.6	22.1	27.5
	4	29.2	13.9	14.9	26.1	30.1

Table A3. Observed Blank Sample Results for Reagent Lot 2 (Units are pg/mL)

Day	Replicate	Blank 1	Blank 2	Blank 3	Blank 4	Blank 5
1	1	4.6	9.2	6.1	4.0	4.0
	2	4.1	8.3	3.2	11.5	6.2
	3	1.6	4.8	3.9	4.5	−0.2
	4	3.7	5.4	1.4	3.6	2.3
2	1	2.2	4.8	3.1	4.4	1.6
	2	0.7	6.3	4.1	6.8	2.6
	3	4.6	5.4	1.0	7.1	6.4
	4	2.6	9.6	3.4	4.2	5.7
3	1	1.1	7.7	0.1	3.7	4.2
	2	−4.4	3.1	0.4	3.7	3.7
	3	0.9	6.1	2.9	5.3	1.4
	4	0.7	10.0	−1.6	4.5	1.5

Table A4. Observed Low level Sample Results for Reagent Lot 2 (Units are pg/mL)

Day	Replicate	Low 1	Low 2	Low 3	Low 4	Low 5
1	1	22.0	15.6	13.0	18.8	32.9
	2	22.5	21.2	15.9	17.6	30.4
	3	21.8	14.8	9.0	14.1	29.4
	4	22.1	14.9	7.0	14.9	27.6
2	1	20.3	16.0	13.4	19.2	27.7
	2	21.0	15.8	8.5	15.8	30.6
	3	25.3	21.6	16.3	19.8	31.4
	4	26.0	22.8	18.1	21.4	30.4
3	1	27.2	15.3	12.4	18.0	32.5
	2	25.1	18.7	11.1	18.0	28.9
	3	25.3	18.3	11.3	19.6	29.8
	4	25.3	19.5	10.1	23.1	35.1

The data were assessed using the nonparametric data analysis option. This is the most common treatment because it makes no assumptions on the underlying data distribution and works equally well with censored and noncensored blank sample measurements.

Because there were two reagent lots, LoB estimates were evaluated separately for each lot, per Section 5.3.3.1 of this document. Measurement results from the five blank samples were combined for a given reagent lot, then sorted from low to high.

Appendix A. (Continued)

Using the typical Type I error risk of $\alpha = 0.05$, the corresponding percentile Pct_B was given by:

$$Pct_B = 1 - \alpha = 0.95 \quad \text{(A1)}$$

The rank position corresponding to this Pct_B percentile was computed as:

$$\text{Rank Position} = 0.5 + (B \bullet Pct_B) = 0.5 + (60 \bullet 0.95) = 57.5 \quad \text{(A2)}$$

where $B = 60$ is the number of blank sample measurements per reagent lot in this example. As rank positions are integers, the value corresponding to a rank position 57.5 was calculated by interpolating between values of the adjacent integral rank positions 57 and 58. This interpolated value represents the LoB estimate. Table A5 contains the upper rank values for the sorted blank measurement results and the resulting LoB estimates of 7.6 and 9.4 pg/mL for reagent lots 1 and 2, respectively. The greater of these values—9.4 pg/mL—was reported as the LoB for the measurement procedure.

Table A5. Rank Positions and LoB From Blank Sample Test Results

Rank Position	Value (Reagent Lot 1)	Value (Reagent Lot 2)
56	7.6	8.3
57	7.6	9.2
58	7.6	9.6
59	8.0	10.0
60	8.7	11.5
LoB:	**7.6**	**9.4**

Abbreviation: LoB, limit of blank.

The low level sample results were analyzed next for each reagent lot, per Section 5.3.3.2 of this document. The calculation table shown in Table A6 gives the individual SDs for each sample. These results were pooled per equation 4 to give SD_L. The c_p multiplier factor was calculated per equation 6 using $L = 60$ total low level sample results and $K = 5$ low level samples. The LoD estimate for each reagent lot was calculated per equation 5, using the reported LoB determined above. This gave results of 14.5 and 13.8 pg/mL for reagent lot 1 and 2, respectively. For this example, the greater of the two estimates—14.5 pg/mL—was reported as the LoD for the measurement procedure.

Table A6. SDs and LoD Calculations From Low Level Sample Test Results

	Reagent Lot 1		Reagent Lot 2	
Sample	**n**	**SD**	**n**	**SD**
Low 1	12	3.15	12	2.27
Low 2	12	2.17	12	2.87
Low 3	12	2.62	12	3.37
Low 4	12	3.61	12	2.59
Low 5	12	3.73	12	2.18
$\boldsymbol{SD_L}$		**3.11**		**2.69**
$\boldsymbol{c_p}$		**1.653**		**1.653**
LoD:		**14.5**		**13.8**

Abbreviations: LoD, limit of detection; SD, standard deviation.

A suggested format for reporting these example results in the product's instructions for use (see Section 8.2 of this document) is:

Appendix A. (Continued)

> "The LoD for estradiol is 14.5 pg/mL, determined consistent with the guidelines in CLSI document EP17 based on the proportions of false positives (α) less than 5% and false negatives (β) less than 5%; using 240 determinations, with 120 blank and 120 low level samples; and an LoB of 9.4 pg/L."

To complete this worked example, the data were reanalyzed to provide an LoB estimate using the parametric data analysis option. This required initial confirmation that the blank sample data follow a normal distribution—an uncommon situation with most datasets, particularly if there is any censoring. For the example data, all 120 blank measurements across the two reagent lots were used to create a composite histogram (see Figure A1).

The shape appeared reasonably normal, an observation further supported by results of a Shapiro-Wilk W test ($p = 0.083$; do not reject hypothesis of a normal distribution). Based on this assessment; it was judged appropriate to move forward with the parametric data analysis option.

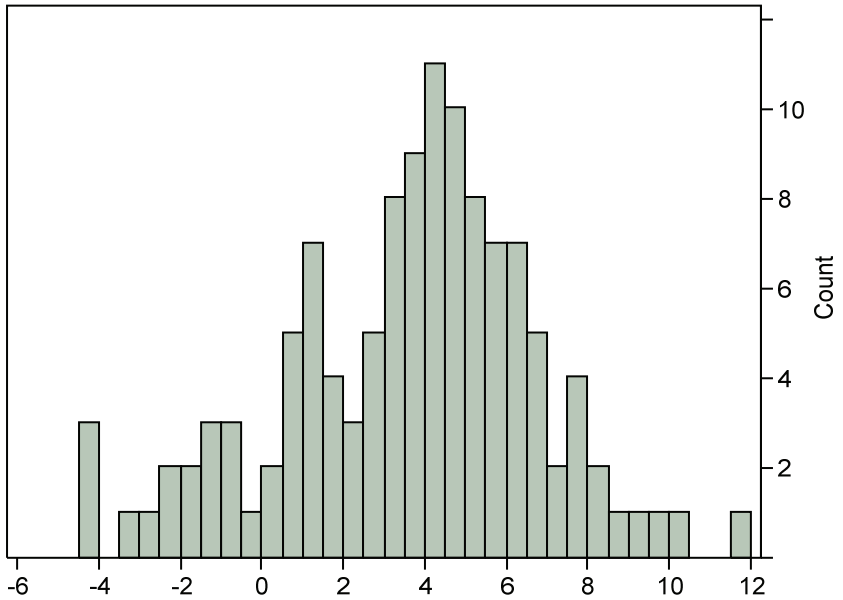


Figure A1. Histogram of Combined Blank Sample Measurements

Per Section 5.3.3.1 of this document, the grand means and SDs were calculated across all blank results for each reagent lot independently. These values, along with the c_p of 1.653 (from $B = 60$ and $K = 5$) yielded LoB estimates of 8.8 and 8.6 pg/mL for reagent lots 1 and 2, respectively. The greater of these values—8.8 pg/mL—was reported as the LoB for the measurement procedure. See Table A7.

Table A7. LoB Calculations Using Parametric Data Analysis Option

	Reagent Lot 1	Reagent Lot 2
M_B	3.15	3.90
SD_B	3.11	2.85
c_p	1.653	1.653
LoB	8.8	8.6

Abbreviation: LoB, limit of blank.

With LoB in hand, the low level sample measurements were reanalyzed as done for the nonparametric option. This yielded LoD estimates of 13.9 and 13.2 pg/mL for reagent lot 1 and 2, respectively. For this example, the greater of the two estimates—13.9 pg/mL—was reported as the LoD for the measurement procedure.

Appendix A. (Continued)

The parametric-derived LoB estimate of 8.8 pg/mL compared well with the 9.4 pg/mL value obtained by the nonparametric approach, due to the relative normality of the blank data in this particular example. Datasets with non-normal distributions may show discrepancies in estimates from the two LoB calculation options and only the nonparametric approach is recommended in such cases.

Appendix B. Worked Example: Evaluation of Limit of Detection by the Precision Profile Approach

Data for this example came from a detection capability study for a new cardiac marker immunoassay. Because initial precision testing results showed repeatability to increase with measurand concentration, the precision profile protocol was used to evaluate the limit of detection (LoD).

Five blank samples were made using immunoadsorption of individual patient serum samples to remove endogenous measurand. These samples were tested using the classical approach design with two reagent lots over three test days, with n = 4 replicates per day per reagent lot. The results were analyzed by the nonparametric option and yielded a limit of blank (LoB) estimate of 0.51 ng/mL.

The experimental design listed below was selected to meet the minimum requirements of Section 5.4.1.1 of this document. It followed the 20-day, two-runs-per-day design described in CLSI document EP05[1] to yield estimates of within-laboratory precision over a large number of days. The default values of $\alpha = \beta = 0.05$ were used for the Type I and II error risks.

- Two reagent lots (one and two)
- One instrument system
- One calibrator and calibration per reagent lot (at the start of the study)
- Six low level samples
- 20 test days, two runs per day
- Two replicate measurements per sample per run for each reagent lot
- Total of 80 measurement values per sample for each reagent lot

The study was run per the above design. Measurements were acquired in analyte values. Table B1 lists the observed mean measurand concentrations and associated within-laboratory precision SDs (SD_{WL}) for the samples, broken out by reagent lot, calculated per CLSI document EP05.[1]

Table B1. Observed Within-Laboratory Precision and Mean Concentration for All Samples

	Reagent Lot 1		Reagent Lot 2	
Sample Identification	**Mean (ng/mL)**	**SD_{WL} (ng/mL)**	**Mean (ng/mL)**	**SD_{WL} (ng/mL)**
A	0.69	0.39	0.78	0.29
B	1.42	0.39	1.73	0.54
C	2.65	0.46	2.89	0.55
D	4.08	0.55	3.82	0.63
E	6.08	0.64	6.33	0.82
F	10.36	1.12	10.92	1.38

Abbreviation: SD, standard deviation.

There were two reagent lots in the study; therefore, separate data analysis was done for each lot. Precision profiles were plotted as shown in Figure B1 and both appeared to follow smooth curves. Although a number of different models could be fit to the data, a second-order polynomial was deemed by visual inspection to show acceptable agreement to the profiles.

Appendix B. (Continued)

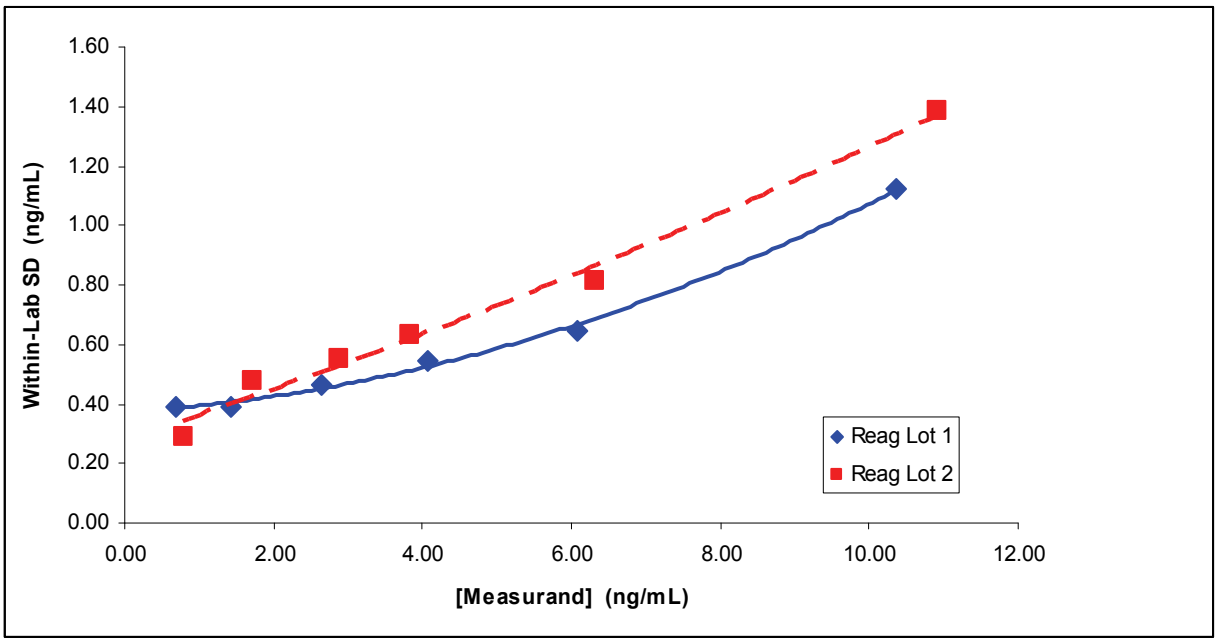


Figure B1. Precision Profiles of Within-Laboratory Precision (SD_{WL}) vs Measurand Concentration With Second-Order Polynomial Model Fit Overlays

The polynomial model regression fits were done using a spreadsheet. The output model coefficients were:

$$SD_{WL} = C_0 + C_1X + C_2X^2 \quad \text{(B1)}$$

$SD_{WL} = 0.3741 + 0.0149\text{X} + 0.0055\text{X}^2$ Reagent Lot 1

$SD_{WL} = 0.2801 + 0.0817\text{X} + 0.0017\text{X}^2$ Reagent Lot 2

Based on these polynomial models, a trial LoD estimate was calculated for each reagent lot using the LoB as a starting point and the following equations (from Section 5.4.3.2 of this document):

$$\text{LoD} = \text{LoB} + c_p SD_{WL} \quad \text{(B2)}$$

$$c_p = \frac{1.645}{1 - \frac{1}{4(N_{TOT} - K)}} \quad \text{(B3)}$$

where N_{TOT} = the total number of measurements (number of measurement results per sample × number of samples) and K = the number of samples.

$$c_p = \frac{1.645}{1 - \frac{1}{4((80 \bullet 6) - 6)}} = \frac{1.645}{1 - \frac{1}{474}} = 1.646$$

$\text{LoD} = 0.51 \text{ ng/mL} + 1.646[0.3741 + 0.0149(0.51) + 0.0055(0.51)^2] = 1.14 \text{ ng/mL}$ Reagent Lot 1

Appendix B. (Continued)

$$\text{LoD} = 0.51 \text{ ng/mL} + 1.646[0.2801 + 0.0817(0.51) + 0.0017(0.51)^2] = 1.04 \text{ ng/mL} \quad \text{Reagent Lot 2}$$

Because the trial LoD values of 1.14 and 1.04 ng/mL did not equal the starting measurand concentration of 0.51 ng/mL, it was necessary to calculate additional trial LoD values by increasing the measurand concentrations until equivalence was reached (ie, zero bias). Equation B2 was applied for each reagent lot sequentially using measurand concentrations of 0.50 through 1.30 ng/mL, incremented by 0.1 ng/mL.

SD_{WL} values were calculated using equation B1 at the corresponding measurand concentrations (MC). The trial LoD values then were calculated using equation B2 and the SD_{WL} values. Bias was calculated by subtracting MC values from the trial LoD values.

The results are summarized in the first section of Table B2 and show that the LoD estimates fall between 1.10 and 1.20 for each reagent lot (identified by noting that the sign of the bias changes between these measurand concentrations). This process was repeated using a smaller step size of 0.01 ng/mL over the interval 1.10–1.20 pg/mL, as shown in the second section of Table B2.

Table B2. Trial LoD Values vs MC

	Reagent Lot 1			Reagent Lot 2		
MC (ng/mL)	***SD_{WL}*** **(ng/mL)**	**Trial LoD (ng/mL)**	**Bias (ng/mL)**	***SD_{WL}*** **(ng/mL)**	**Trial LoD (ng/mL)**	**Bias (ng/mL)**
0.50	0.383	1.14	0.64	0.348	1.08	0.58
0.60	0.385	1.14	0.54	0.355	1.10	0.50
0.70	0.387	1.15	0.45	0.363	1.11	0.41
0.80	0.390	1.15	0.35	0.371	1.12	0.32
0.90	0.392	1.16	0.26	0.379	1.13	0.23
1.00	0.395	1.16	0.16	0.387	1.15	0.15
1.10	0.397	1.16	0.06	0.394	1.16	0.06
1.20	0.400	1.17	−0.03	0.402	1.17	−0.03
1.30	0.403	1.17	−0.13	0.410	1.19	−0.11
1.10	0.397	1.16	0.06	0.394	1.16	0.06
1.11	0.397	1.16	0.05	0.395	1.16	0.05
1.12	0.398	1.16	0.04	0.396	1.16	0.04
1.13	0.398	1.17	0.04	0.397	1.16	0.03
1.14	0.398	1.17	0.03	0.398	1.16	0.02
1.15	0.399	1.17	0.02	0.398	1.17	0.02
1.16	0.399	1.17	0.01	0.399	1.17	0.01
1.17	0.399	1.17	0.00	0.400	1.17	0.00
1.18	0.399	1.17	−0.01	0.401	1.17	−0.01
1.19	0.400	1.17	−0.02	0.401	1.17	−0.02
1.20	0.400	1.17	−0.03	0.402	1.17	−0.03

Abbreviations: LoD, limit of detection; MC, measurand concentration; SD, standard deviation.

Inspection of the results shows that a measurand concentration of 1.17 ng/mL yielded a matching trial LoD of 1.17 ng/mL for reagent lot 1 (shaded cells). Similarly, the LoD for reagent lot 2 also was identified as 1.17 pg/mL. For this example, the two estimates were equal and 1.16 ng/mL was reported as the LoD for the measurement procedure.

Appendix B. (Continued)

Reference for Appendix B

[1] CLSI/NCCLS. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition*. CLSI/NCCLS document EP05-A2. Wayne, PA: NCCLS; 2004.

Appendix C. Worked Example: Evaluation of Limit of Detection by the Probit Approach

This example is for a molecular diagnostics test for which the limit of blank (LoB) equals zero. Data were collected using three lots of reagent to establish the limit of detection (LoD) by probit analysis for a microbiology test done on bacterial DNA. A single patient sample is used to simplify this example, rather than the minimum of three as required by the protocol. A dilution series of five measurand concentrations was prepared from the sample and a set of measurement replicates were made for each dilution using three reagent lots. Also, a negative pool was prepared from native specimens and tested with replication to demonstrate that LoB = 0. The number of positive results observed, total number of measurements made, and calculated hit rate ratios are summarized in Table C1 for each experimental condition.

Table C1. Observed Proportions of Positive Test Results With the Planned Dilutions

Concentration (CFU/mL)	Observed Positive/Total Results			Hit Rate		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
0.000	0/22	0/22	0/22	0.000	0.000	0.000
0.025	23/32	28/32	27/32	0.719	0.875	0.844
0.050	29/32	32/32	32/32	0.906	1.000	1.000
0.150	32/32	32/32	32/32	1.000	1.000	1.000
0.300	32/32	32/32	32/32	1.000	1.000	1.000
0.500	32/32	32/32	32/32	1.000	1.000	1.000

Abbreviation: CFU, colony-forming unit.

The results from these planned concentration levels show only one or two nonzero levels with a hit rate less than 1.000. Such data do not allow for fitting probit models. For this reason, two additional, lower concentration levels were tested and added to the initial data. The results are shown in Table C2.

Table C2. Observed Proportions of Positive Test Results With Additional Dilutions

Concentration (CFU/mL)	Observed Positive/Total Results			Hit Rate		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
0.006	11/30	12/30	22/34	0.367	0.400	0.647
0.014	15/30	22/30	31/34	0.500	0.733	0.912

Abbreviation: CFU, colony-forming unit.

The data were reanalyzed using software for probit analysis. This approach allows use of all data obtained with low level samples and provides associated confidence intervals for the LoD estimates. No lack of fit of the probit models was detected, using both a Pearson chi-square test and a log-likelihood ratio chi-square test, which allowed for prediction of the LoD. The results are summarized in Table C3 and the graphs of fitted probit models, including 95% confidence bands for the respective fits, are shown in Figures C1, C2, and C3.

Table C3. Summaries of the Probit Analysis Results

	Lot 1	Lot 2	Lot 3
LoD (CFU/mL)	0.077	0.033	0.031

Abbreviation: CFU, colony forming unit.

The maximum estimate of 0.077 CFU/mL (reagent lot 1) was reported as the LoD for the measurement procedure.

Appendix C. (Continued)

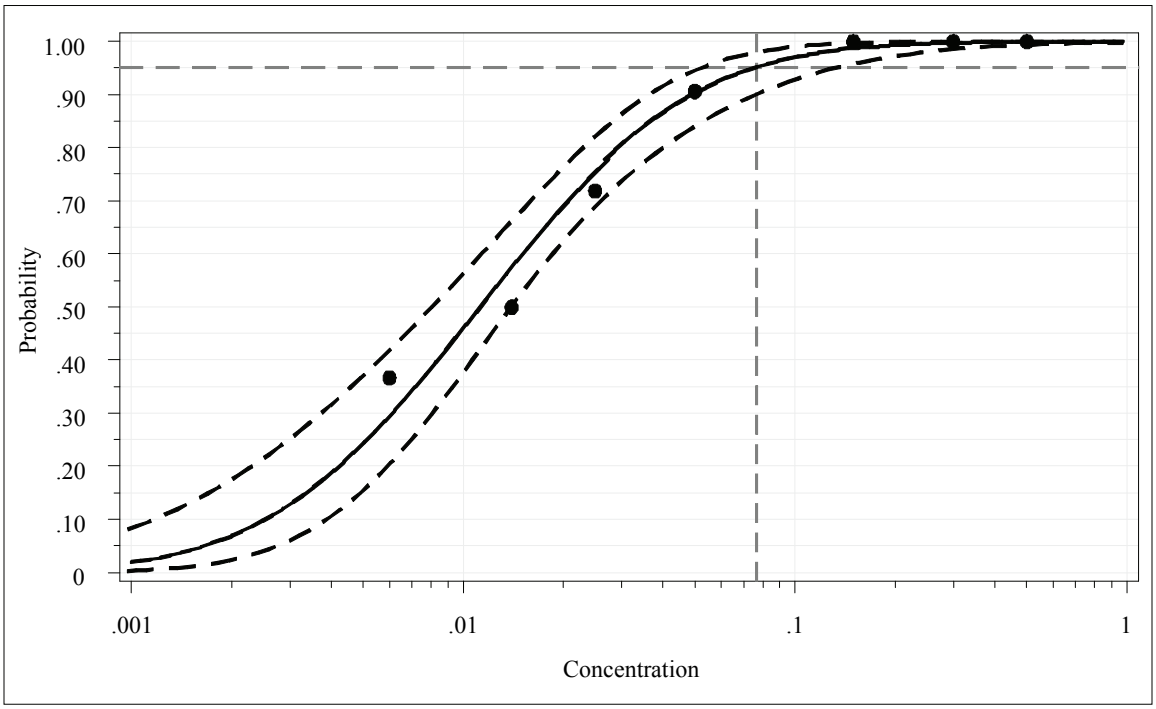


Figure C1. Probit Analysis for Reagent Lot 1

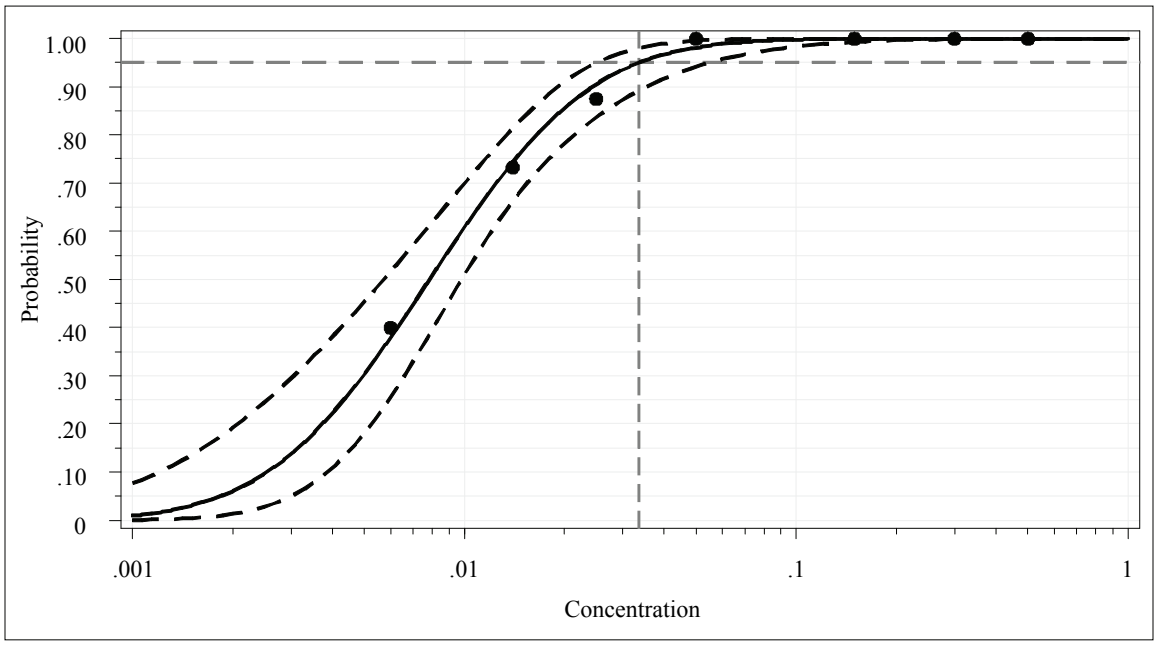


Figure C2. Probit Analysis for Reagent Lot 2

Appendix C. (Continued)

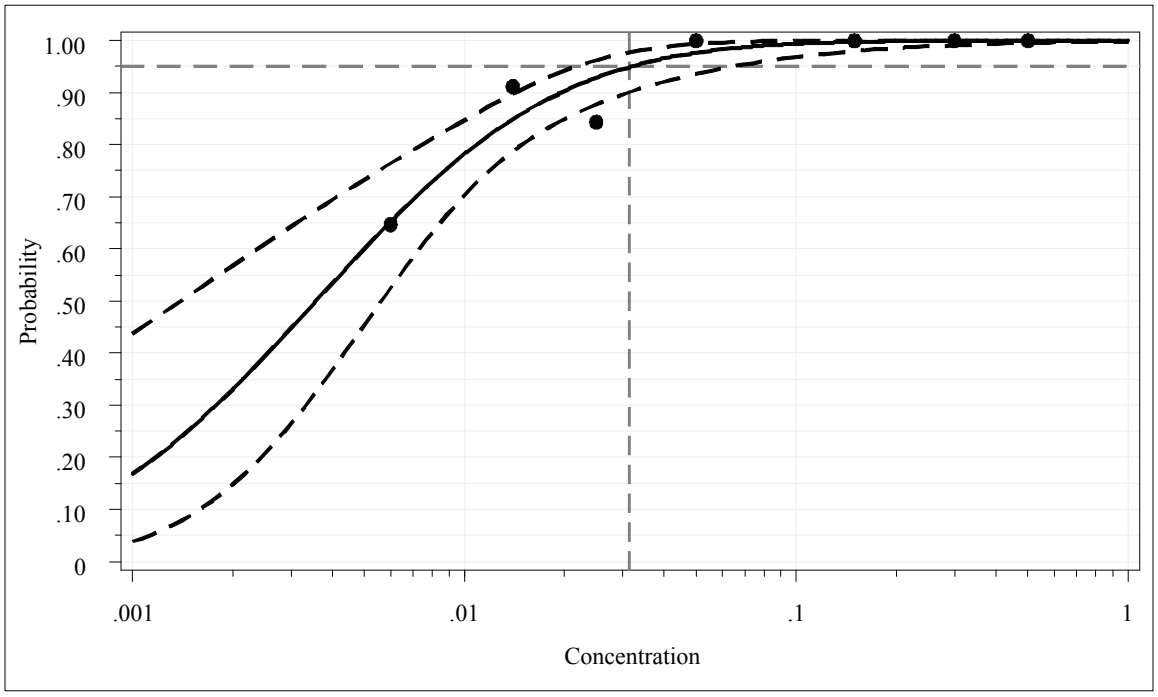


Figure C3. Probit Analysis for Reagent Lot 3

Appendix D. Worked Examples: Evaluation of Limit of Quantitation

D1 Example 1: Limit of Quantitation as Functional Sensitivity

Functional sensitivity has been used as a detection capability performance attribute for cardiac, cancer, and thyroid measurement procedures. It represents the measurand concentration associated with a desired within-laboratory precision, based upon a precision profile experiment in the low-end region of the measuring interval. This performance attribute, however, simply represents a limiting form of the LoQ in which the acceptable accuracy goal is based solely upon a precision requirement.

For this example, the LoQ was evaluated for a cardiac troponin I measurement procedure. The accuracy goal was defined as within-laboratory precision equal to 10 percent CV (%CV), using the 20-day protocol within CLSI document EP05[1] to estimate within-laboratory precision. The experimental design met the minimum requirements listed in Section 6.3 of this document, with two reagent lots, one instrument system, nine low level serum pool samples, 20 testing days, two runs per day, and two replicates per run (total of 80 replicates per sample).

The two reagent lots A and B were calibrated at the start of the study. Nine serum pools were prepared from native human sera at nominal concentrations across the low-end region of the measuring interval. The pools were aliquotted and frozen at −70°C before starting the study. Data were collected over 20 consecutive working days, by testing freshly thawed aliquots of each serum pool sample in a randomized order, each run with both reagent lots. Estimates of the mean and within-laboratory precision (SD_{WL}) were calculated for each sample by each reagent lot. These are tabulated in Table D1 and plotted as precision profiles in Figure D1 for each reagent lot.

Table D1. Summary of the Observed Precision Estimates

	Reagent Lot 1			Reagent Lot 2		
Sample Pool Identification	**Mean (ng/mL)**	**SD_{WL} (ng/mL)**	**CV (%)**	**Mean (ng/mL)**	**SD_{WL} (ng/mL)**	**CV (%)**
Pool 1	0.040	0.016	40.2	0.041	0.018	44.1
Pool 2	0.053	0.016	29.6	0.047	0.014	28.8
Pool 3	0.080	0.016	19.5	0.077	0.012	15.1
Pool 4	0.111	0.017	15.1	0.106	0.019	17.8
Pool 5	0.137	0.014	10.0	0.136	0.016	11.4
Pool 6	0.164	0.012	7.4	0.159	0.015	9.2
Pool 7	0.190	0.011	6.0	0.182	0.015	8.4
Pool 8	0.214	0.016	7.5	0.205	0.016	7.8
Pool 9	0.245	0.013	5.4	0.234	0.014	6.2

Abbreviations: CV, coefficient of variation; SD, standard deviation.

Appendix D. (Continued)

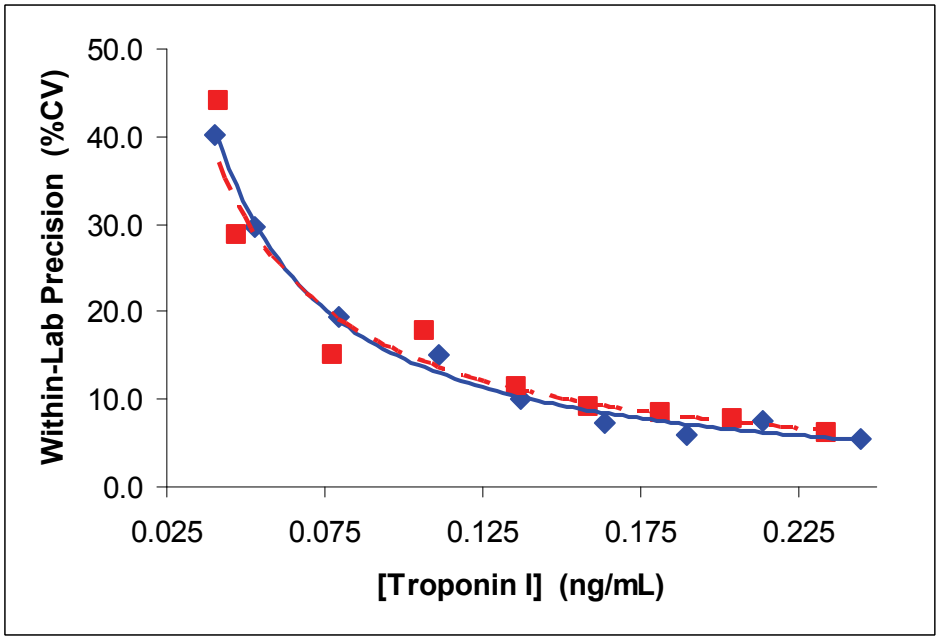


Figure D1. Precision Profiles of Within-Laboratory Precision (as %CV) vs Measurand Concentration With a Power Function Model Fit Overlays. The LoQ estimate for each reagent lot was determined as the measurand concentration at the intersection of its power function model fit line with the accuracy goal of a 10 %CV.

Based upon the shape of the precision profiles, a power function model was used to fit the datasets.

$$\%\mathrm{CV} = C_0 X^{C_1} \tag{D1}$$

The power function model parameters were estimated for each reagent lot using regression analysis. The results were $C_0 = 1.0937$, $C_1 = -1.128$ for Reagent Lot 1 and $C_0 = 1.5118$, $C_1 = -1.0033$ for Reagent Lot 2. Solving equation D1 to find X for the desired Y = 10 %CV accuracy goal yielded:

$$X = \left(\frac{Y}{C_0}\right)^{1/C_1} = \left(\frac{10}{1.0937}\right)^{-1/1.128} = 0.14 \text{ ng/mL for Reagent Lot 1} \tag{D2}$$

$$X = \left(\frac{Y}{C_0}\right)^{1/C_1} = \left(\frac{10}{1.5118}\right)^{-1/1.0033} = 0.15 \text{ ng/mL for Reagent Lot 2} \tag{D3}$$

The solutions also can be determined directly from the plot as the concentration at the intersection of each precision profile curve with the accuracy goal of 10%. The maximum value of 0.15 ng/mL (reagent lot 2) was reported as the LoQ for the measurement procedure.

Appendix D. (Continued)

D2 Example 2: Limit of Quantitation Based Upon Total Error

This example evaluates the LoQ for the immunometric estradiol measurement procedure that was used in the limit of blank/limit of detection (LoB/LoD) example in Appendix A of this document. The LoQ accuracy goal is a total error (TE) of 21.6%, based upon the desirable TE specification from the biological variation database provided by C. Ricos, et al.[2] The classical Westgard model is used to define TE for this evaluation:

$$TE = |Bias| + 2s \quad \text{(D4)}$$

Data from LoB/LoD testing in Appendix A were used to guide an initial estimate of the LoQ. The testing in Appendix A used the following experimental design, which met the minimum requirements in Section 6.3 of this document.

- Two reagent lots (A and B)
- One instrument system
- Three test days with four replicate measurements per sample for each reagent-day combination
- Five low level samples
- 60 total low level sample measurements per reagent lot
  (3 days × 5 samples × 4 replicates)

Table D2 summarizes the observed means and SDs from the individual low level sample measurements given in Appendix A. Aliquots of each low level sample were analyzed by an isotope dilution-gas chromatography/mass spectrometry (ID-GC/MS) reference measurement procedure to give the reference values listed in the table.

Table D2. Observed Means and SDs for Low Level Samples From Appendix A Data

	Reference Value	Observed Mean (pg/mL)		Observed SD (pg/mL)	
Sample	(pg/mL)	Reagent A	Reagent B	Reagent A	Reagent B
Low 1	26.1	25.0	23.7	3.1	2.3
Low 2	16.9	14.2	17.9	2.2	2.9
Low 3	13.1	13.9	12.2	2.6	3.4
Low 4	20.4	21.6	18.4	3.6	2.6
Low 5	27.8	25.1	30.6	3.7	2.2

Abbreviation: SD, standard deviation.

For each reagent lot, bias was calculated for each low level sample as the observed mean minus the reference value. This was combined with the observed SD, per equation D4, to yield TE, which was expressed as a percentage of the associated sample reference value. See Table D3.

Appendix D. (Continued)

Table D3. TE Calculations for Low Level Samples From Appendix A Data

	Reference Value	Bias (pg/mL)		TE (%)		
Sample	(pg/mL)	Reagent A	Reagent B	Reagent A	Reagent B	Average
Low 1	26.1	−1.1	−2.4	28.3%	26.6%	27.5%
Low 2	16.9	−2.7	1.0	41.7%	39.9%	40.8%
Low 3	13.1	0.8	−0.9	46.1%	58.3%	52.2%
Low 4	20.4	1.2	−1.0	41.2%	30.3%	35.8%
Low 5	27.8	−2.7	2.8	36.5%	25.7%	31.1%

Abbreviation: TE, total error.

Visual inspection of these results shows that none of the low level samples yielded a TE meeting the accuracy goal of 21.6%, although performance did improve with increasing measurand concentration.

Extrapolating from a plot of the average TE vs the reference value (see Figure D2) suggests that the accuracy goal might be achieved around 35 pg/mL.

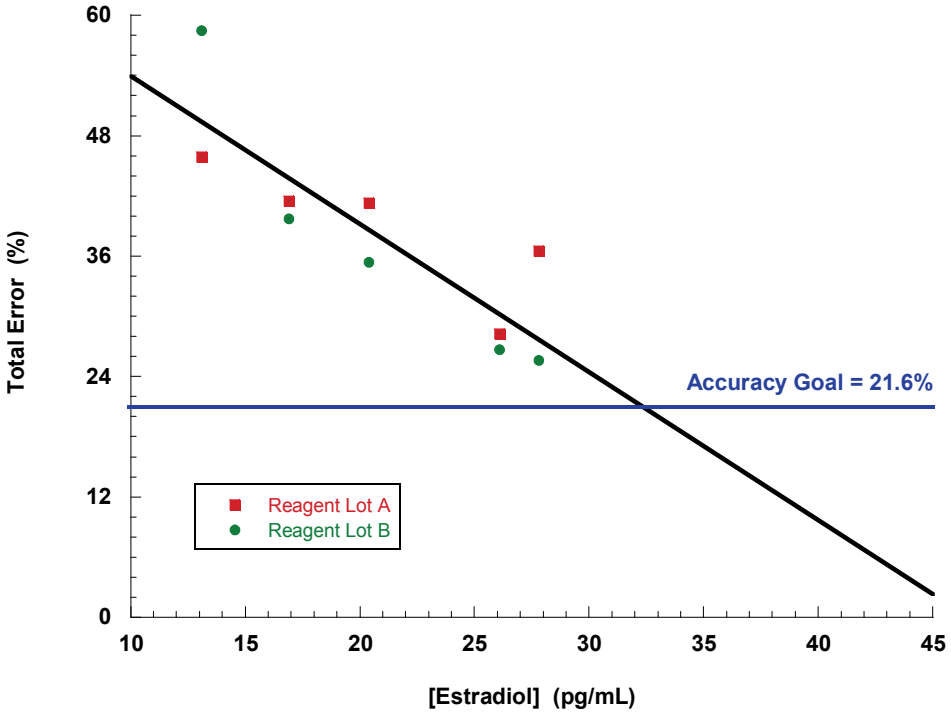


Figure D2. Plot of Calculated TE vs Reference Value for Low Level Samples by Two Reagent Lots, With Linear Regression Model Fit and Extrapolation

Based upon this result, five pooled samples were prepared at a nominal target measurand concentration of 40 pg/mL. The samples were analyzed with an ID-GC/MS reference measurement procedure to assign reference values and confirm that they were in an acceptable range of the desired measurand concentration range (40 ± 10 pg/mL). Each of the five samples was tested in triplicate by each of two reagent lots over each of three test days using a single instrument system. This yielded 45 replicates per reagent lot. The observed results and reference values for the samples are summarized below in Tables D4 and D5.

Appendix D. (Continued)

Table D4. Observed Results for Reagent Lot A

		Pool 1	Pool 2	Pool 3	Pool 4	Pool 5
	Reference Values	38.2	47.1	44.7	36.5	42.8
Day	**Replicate**					
1	1	36.7	49.9	46.1	33.3	42.9
1	2	37.9	50.0	43.1	34.2	41.8
1	3	38.3	48.1	39.4	34.5	43.8
2	1	36.8	47.8	47.3	43.1	46.3
2	2	33.5	43.9	45.8	34.0	43.3
2	3	39.2	45.6	44.8	37.1	46.0
3	1	41.3	45.4	44.6	35.3	42.6
3	2	37.9	51.5	47.3	32.4	41.4
3	3	34.9	45.8	38.9	36.0	42.8

Table D5. Observed Results for Reagent Lot B

		Pool 1	Pool 2	Pool 3	Pool 4	Pool 5
	Reference Values	38.2	47.1	44.7	36.5	42.8
Day	**Replicate**					
1	1	38.5	45.8	46.7	35.5	42.0
1	2	41.0	47.8	43.6	40.0	44.1
1	3	43.2	46.6	42.4	34.0	43.2
2	1	36.8	46.9	46.5	32.9	46.6
2	2	42.1	51.3	47.9	33.1	45.5
2	3	35.8	50.5	42.7	38.6	43.5
3	1	36.8	44.3	42.1	36.2	41.4
3	2	44.1	47.5	43.4	41.4	48.2
3	3	39.5	52.4	44.7	33.0	45.7

The mean, SD, and bias relative to the reference values were calculated for each sample per reagent lot. These values were used to calculate the TE by equation D4 for each sample, which were expressed as %TE relative to the respective reference value for each sample. The calculations are summarized in Table D6.

Appendix D. (Continued)

Table D6. LoQ Intermediate Calculations for Reagent Lots A and B

Sample	Reference Value	Mean (pg/mL)	SD (pg/mL)	Bias (pg/mL)	TE (pg/mL)	TE (%)
Pool 1	38.2	37.4	2.3	−0.8	5.4	14.1
Pool 2	47.1	47.6	2.6	0.5	5.7	12.1
Pool 3	44.7	44.1	3.1	−0.6	6.8	15.2
Pool 4	36.5	35.5	3.2	−1.0	7.4	20.3
Pool 5	42.8	43.4	1.7	0.6	4.0	9.3
					LoQ for Reagent Lot A	35.5 pg/mL
Pool 1	38.2	39.8	3.0	1.6	7.6	19.9
Pool 2	47.1	48.1	2.7	1.0	6.4	13.6
Pool 3	44.7	44.4	2.1	−0.3	4.5	10.1
Pool 4	36.5	36.1	3.2	−0.4	6.8	18.6
Pool 5	42.8	44.5	2.2	1.7	6.1	14.3
					LoQ for Reagent Lot B	36.1 pg/mL

Abbreviations: LoQ, limit of quantitation; SD, standard deviation; TE, total error.

The calculated TE for all samples by each reagent lot meets the accuracy goal of %TE ≤21.6%; therefore, the LoQ criterion is deemed to be met. The lowest sample concentration that met the accuracy goal for Reagent Lot A is 35.5 pg/mL. For Reagent Lot B, the LoQ is 36.1 pg/mL.The greater of these two estimates, 36.1 pg/mL, is reported as the LoQ for the measurement procedure.

References for Appendix D

[1] CLSI/NCCLS. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.* CLSI/NCCLS document EP05-A2. Wayne, PA: NCCLS; 2004.

[2] Ricos C, Iglesias N, Garcia-Lario JV, et al. Within-subject biological variation in disease: collated data and clinical consequences. *Ann Clin Biochem.* 2007;44:343-352. http://www.westgard.com/biological-variation-in-patients-with-disease.htm. Accessed May 14, 2012.

Appendix E. Worked Example: Verification of Limits of Blank and Detection Claims

A manufacturer claims that its measurement procedure for a drug has a limit of blank (LoB) of 20 ng/mL and a limit of detection (LoD) of 45 ng/mL, with error risks $\alpha = \beta = 0.05$. The user wants to verify both detection capability claims.

For the LoB verification, two blank patient samples (no drug) were tested in replicates of $n = 4$ each day for three days. For LoD verification, separate aliquots of these same blank samples were spiked to a nominal measurand concentration of 45 ng/mL, then also measured in $n = 4$ replicates on each of the same three testing days. A single reagent lot and instrument system was used to acquire all data (a total of 24 measurements for each of the blank and low level samples). The testing results were sorted by increasing measurand concentration and are listed in Table E1.

Table E1. Observed Blank and Positive Results for LoB/LoD Verification (Units are ng/mL)

Rank	Blanks	Positives
1	0.00	18.80
2	0.00	19.02
3	0.00	26.63
4	0.00	26.91
5	0.00	31.08
6	0.00	33.99
7	0.00	35.11
8	0.00	35.90
9	1.08	41.67
10	1.92	43.90
11	2.38	46.32
12	2.98	47.77
13	3.80	47.99
14	4.78	48.83
15	7.30	54.67
16	8.81	57.30
17	10.31	59.10
18	11.29	61.17
19	13.48	61.96
20	14.39	62.97
21	16.97	66.44
22	17.40	73.44
23	18.01	73.80
24	22.65	75.71

Comparison of the blank sample results with the manufacturer's LoB claim of 20 ng/mL showed that all but one result (22.65 ng/mL) were less than or equal to the claim. This gave a percentage of 23/24 = 95.8%. Because this observed percentage exceeded the minimum percentage of 87% in Table 1 (see Section 7.1 of this document) for a 95% confidence interval with a sample size of 24, the manufacturer's LoB claim was deemed to be verified. The Table 1 entry was selected as the greater of the bracketing N values (20 and 30) about the verification study size of $N = 24$.

Similarly, the percentage of positive results that were greater than or equal to the LoB claim was calculated to be 22/24 = 91.7%. Comparison of this percentage with the Table 1 minimum percentage of 87% (per the rationale given above), showed that the observed value exceeded the Table 1 value; thus, the manufacturer's LoD claim was deemed to be verified.

Appendix F. Worked Example: Verification of Limit of Quantitation Claim

A manufacturer claims that its measurement procedure for a given drug has a limit of quantitation (LoQ) of 50 pg/mL, based upon an accuracy goal of 15% total error (TE). The user wants to verify the LoQ, assuming 5% Type I and Type 2 error risks ($\alpha = \beta = 0.05$).

For the LoQ verification, a set of five blank patient samples (no drug) were spiked with drug to a nominal concentration of 50 pg/mL. These samples were tested by a reference measurement procedure (with a known LoQ < 50 pg/mL) to yield target values. Upper and lower allowable TE windows were calculated for each sample as the target value ± 15%. The samples were tested in replicates of n = 3 each day for three days, using a single reagent lot and instrument system to acquire all data (nine results per sample; 45 results total). At the end of testing, the results for each sample were compared to their respective allowable TE window limits and the number of results falling outside of the window was counted. The testing results, target values, allowable error window limits, and number of results outside the window per sample are listed in Table F1.

Table F1. Observed Results for LoQ Verification (Units are pg/mL). Results outside the allowable TE window are shown in bold.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Target Value	46.4	45.8	49.1	46.3	49.7
Error Window					
Lower Limit	39.4	38.9	41.7	39.4	42.2
Upper Limit	53.4	52.7	56.5	53.2	57.2
Test Day					
1	47.8	47.3	49.7	50.4	53.7
2	44.6	48.8	51.2	49.5	52.7
3	47.1	47.6	**57.3**	44.0	55.9
1	50.8	**54.7**	54.6	51.5	55.1
2	48.2	50.7	49.3	51.4	55.5
3	52.5	50.8	53.3	49.8	**57.3**
1	49.4	52.5	**58.0**	46.1	51.8
2	52.0	50.4	49.5	45.7	48.8
3	46.3	49.6	52.2	50.9	51.7
# Outliers	0	1	2	0	1

Four results fell outside of the allowable TE window, for an observed percentage of results meeting the accuracy goal of 41/45 = 91%. From Table 1 (see Section 7.1 of this document), the minimum percentage for a sample size of 45 is 88% (the maximum Table 1 value for the bracketing rows of N = 40 and N = 50). Because the observed percentage exceeded this minimum, the manufacturer's LoQ claim was deemed to be verified.

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 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 as follows:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

EP17-A2 addresses the QSE 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.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
		M29				X C51 EP05 EP06 EP07 EP12 EP14 EP15 MM03				MM03	EP07

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

EP17-A2 does not address any of the clinical laboratory path of workflow processes indicated in the grid below. For a description of the document 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
	MM03	MM03	MM03	MM03	MM03		MM03	

Related CLSI Reference Materials*

C51-A **Expression of Measurement Uncertainty in Laboratory Medicine; Approved Guideline (2012).** This guideline describes a practical approach to assist clinical laboratories in developing and calculating useful estimates of measurement uncertainty, and illustrates their application in maintaining and improving the quality of measured values used in patient care. A CLSI-IFCC joint project.

EP05-A2 **Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers' precision performance claims and determining when such comparisons are valid; as well as manufacturers' guidelines for establishing claims.

EP06-A **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (2003).** This document provides guidance for characterizing the linearity of a method during a method evaluation; for checking linearity as part of routine quality assurance; and for determining and stating a manufacturer's claim for linear range.

EP07-A2 **Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition (2005).** This document provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interfering substances on clinical chemistry test results.

EP12-A2 **User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition (2008).** This document provides a consistent approach for protocol design and data analysis when evaluating qualitative diagnostic tests. Guidance is provided for both precision and method-comparison studies.

EP14-A2 **Evaluation of Matrix Effects; Approved Guideline—Second Edition (2005).** This document provides guidance for evaluating the bias in analyte measurements that is due to the sample matrix (physiological or artificial) when two measurement procedures are compared.

EP15-A2 **User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition (2006).** This document describes the demonstration of method precision and trueness for clinical laboratory quantitative methods utilizing a protocol designed to be completed within five working days or less.

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.

MM03-A2 **Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline—Second Edition (2006).** This guideline addresses topics relating to clinical applications, amplified and nonamplified nucleic acid methods, selection and qualification of nucleic acid sequences, establishment and evaluation of test performance characteristics, inhibitors, and interfering substances, controlling false-positive reactions, reporting and interpretation of results, quality assurance, regulatory issues, and recommendations for manufacturers and clinical laboratories.

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Fundacão Faculdade de Medicina (SP, Brazil)
Gamma-Dynacare Laboratories (ON, Canada)
Garden City Hospital (MI)
Garfield Medical Center (CA)
Gaston Memorial Hospital (NC)
Geisinger Medical Center (PA)
General Directorate of Health Affairs in Riyad Region (Saudi Arabia)
Genesis Healthcare System (OH)
George Washington University Hospital (DC)
Gestión de Calidad (Argentina)
Ghent University Hospital (Belgium)
Good Shepherd Medical Center (TX)
Grana S.A. (TX)
Grand River Hospital (ON, Canada)
Grey Bruce Regional Health Center (ON, Canada)
Group Health Cooperative (WA)
Gundersen Lutheran Medical Center (WI)
Guthrie Clinic Laboratories (PA)
Halton Healthcare Services (ON, Canada)
Hamad Medical Corp-DLMP LAB QM (Qatar)
Hamad Medical Corporation (Qatar)
Hamilton Regional Laboratory Medicine Program - St. Joseph's (ON, Canada)
Hanover General Hospital (PA)
Harford Memorial Hospital (MD)
Harris Methodist Fort Worth (TX)
Harris Methodist Hospital Southwest (TX)
Hartford Hospital (CT)
Health Network Lab (PA)
Health Sciences North (ON, Canada)
Health Sciences Research Institute (Japan)
Health Waikato (New Zealand)
Heartland Health (MO)
Heidelberg Army Hospital (AE)
Helen Hayes Hospital (NY)
Helix (Russian Federation)
Henry Ford Hospital (MI)
Henry M. Jackson Foundation for the Advancement of Military Medicine-MD (MD)
Hi-Desert Medical Center (CA)
Highlands Medical Center (AL)
HJF Naval Infectious Diseases Diagnostic Laboratory (MD)
Hoag Memorial Hospital Presbyterian (CA)
Holy Cross Hospital (MD)
Holy Name Hospital (NJ)
Holy Spirit Hospital (PA)
Hôpital de la Cité-de-La-Santé De Laval (Quebec, Canada)
Hôpital du Haut-Richelieu (PQ, Canada)
Hôpital Maisonneuve-Rosemont (PQ, Canada)
Hôpital Santa Cabrini Ospedale (PQ, Canada)
Horizon Health Network (N.B., Canada)
Hospital Albert Einstein (SP, Brazil)
Hospital Sacre-Coeur de Montreal (Quebec, Canada)
Hôtel-Dieu Grace Hospital Library (ON, Canada)
Howard University Hospital (DC)
Hunter Area Pathology Service (Australia)
Hunter Labs (CA)
Imelda Hospital (Belgium)
Indian River Memorial Hospital (FL)
Indiana University Health Bloomington Hospital (IN)
Indiana University Health Care-Pathology Laboratory (IN)
Indiana University Health Morgan Hospital (IN)
Inova Central Laboratory (VA)
Institut fur Stand. und Dok. im Med. Lab. (Germany)
Institut National de Santé Publique Du Quebec Centre de Doc. - INSPQ (PQ, Canada)
Institute Health Laboratories (PR)
Institute of Clinical Pathology and Medical Research (Australia)
Institute of Laboratory Medicine Landspitali Univ. Hospital (Iceland)
Institute of Medical & Veterinary Science (SA, Australia)
Integrated Regional Laboratories (HCA) (FL)
Interim LSU Hospital/ Med. Center of La (LA)
Intermountain Health Care Lab Services (UT)
International Health Management Associates, Inc. (IL)
International Medical Labs, Inc. (FL)
Jackson County Memorial Hospital (OK)
Jackson Memorial Hospital (FL)
Jackson Purchase Medical Center (KY)
Jessa Ziekenhuis VZW (Belgium)
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John C. Lincoln Hospital - N.MT. (AZ)
John F. Kennedy Medical Center (NJ)
John H. Stroger, Jr. Hospital of Cook County (IL)
John Muir Health (CA)
John T. Mather Memorial Hospital (NY)
Johns Hopkins Medical Institutions (MD)
Johns Hopkins University (MD)
Johnson City Medical Center Hospital (TN)
JPS Health Network (TX)
Kailos Genetics (AL)
Kaiser Permanente (MD)
Kaiser Permanente (OH)
Kaiser Permanente Medical Care (CA)
Kaohsiun Chang Gung Memorial Hospital (Taiwan)
Keelung Chang Gung Memorial Hospital (DC, Taiwan)
Kenora-Rainy River Reg. Lab. Program (ON, Canada)
KFMC (Saudi Arabia)
King Abdulaziz Hospital, Al Ahsa Dept. of Pathology & Laboratory Medicine (Al-hasa, Saudi Arabia)
King Fahad National Guard Hospital KAMC - NGHA (Saudi Arabia)
King Fahad Specialist Hospital-Dammam, K.S.A. (Eastern Region, Saudi Arabia)
King Hussein Cancer Center (Jordan)
King's Daughters Medical Center (KY)
Kingston General Hospital (ON, Canada)
Laboratória Emílio Ribas (CE, Brazil)
Laboratory Alliance of Central New York (NY)
Laboratory Corporation of America (NJ)
Laboratory Medicin Dalarna (Dalarna, Sweden)
LabPlus Auckland District Health Board (New Zealand)
LAC/USC Medical Center (CA)
Lafayette General Medical Center (LA)
Lakeland Regional Medical Center (FL)
Lancaster General Hospital (PA)
Landstuhl Regional Medical Center (Germany)
Langley Air Force Base (VA)
LeBonheur Children's Hospital (TN)
Legacy Laboratory Services (OR)
Letherbridge Regional Hospital (AB, Canada)
Lewis-Gale Medical Center (VA)
Lexington Medical Center (SC)
L'Hotel-Dieu de Quebec (PQ, Canada)
Licking Memorial Hospital (OH)
LifeBridge Health Sinai Hospital (MD)
LifeLabs Medical Laboratory Services (BC, Canada)
Lifeline Hospital (United Arab Emirates)
Loma Linda University Medical Center (LLUMC) (CA)
Long Beach Memorial Medical Center-LBMMC (CA)
Long Island Jewish Medical Center (NY)
Longview Regional Medical Center (TX)
Louisiana Office of Public Health Laboratory (LA)
Louisiana State University Medical Ctr. (LA)
Lower Columbia Pathologists, P.S. (WA)
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Lyndon B. Johnson General Hospital (TX)
Maccabi Medical Care and Health Fund (Israel)
Madigan Army Medical Center (WA)
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Main Line Clinical Laboratories, Inc. Lankenau Hospital (PA)
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Marquette General Hospital (MI)
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Martin Luther King, Jr./Drew Medical Center (CA)
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Mary Washington Hospital (VA)
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Mater Health Services - Pathology (Australia)
Maxwell Air Force Base (AL)
Mayo Clinic (MN)
McDonald Army Health Center (VA)
MCG Health (GA)
Meadows Regional Medical Center (GA)
Medecin Microbiologiste (Quebec, Canada)
Medical Center Hospital (TX)
Medical Centre Ljubljana (Slovenia)
Medical College of Virginia Hospital (VA)
Medical University Hospital Authority (SC)
Memorial Hermann Healthcare System (TX)
Memorial Hospital at Gulfport (MS)
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Methodist Willowbrook Hospital (TX)
MetroHealth Medical Center (OH)
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Middlesex Hospital (CT)
Minneapolis Medical Research Foundation (MN)
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Mississippi Public Health Lab (MS)
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National Cancer Center (Korea, Republic Of)
National Institutes of Health, Clinical Center (MD)
National Naval Medical Center (MD)

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New England Fertility Institute (CT)
New England Sinai Hospital (MA)
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New York University Medical Center (NY)
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Northside Medical Center (OH)
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Northwestern Memorial Hospital (IL)
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Ohio Valley Medical Center (WV)
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Orebro University Hospital (Sweden)
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Ospedale Casa Sollievo Della Sofferenza - IRCCS (Italy)
Our Lady's Hospital For Sick Children (Ireland)
Palmetto Baptist Medical Center (SC)
Pamela Youde Nethersole Eastern Hospital (Hong Kong East Cluster) (Hong Kong)
Pathgroup (TN)
Pathlab (IA)
Pathology and Cytology Laboratories, Inc. (KY)
Pathology Associates Medical Lab. (WA)
Pathology Inc. (CA)
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Potomac Hospital (VA)
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Prince of Wales Hospital (Hong Kong)
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Queensway Carleton Hospital (ON, Canada)
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St. Francis Memorial Hospital (CA)
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St. Luke's Hospital (IA)
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St. Mary's Good Samaritan (IL)
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VA (Indianapolis) Medical Center (IN)
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Virginia Regional Medical Center (MN)
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Walter Sisulu University (EC, South Africa)
Warren Hospital (NJ)
Washington Hospital Center (DC)
Washington Hospital Healthcare System (CA)
Waterbury Hospital (CT)
Weed Army Community Hospital Laboratory (CA)
Weirton Medical Center (WV)
West Jefferson Medical Center (LA)
West Penn Allegheny Health System-Allegheny General Hospital (PA)
West Shore Medical Center (MI)
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Westchester Medical Center (NY)
Western Baptist Hospital (KY)
Western Healthcare Corporation (NL, Canada)
Wheaton Franciscan Laboratories (WI)
Wheeling Hospital (WV)
White Memorial Medical Center (CA)
Whitehorse General Hospital (YT, Canada)
William Beaumont Army Medical Center (TX)
William Beaumont Hospital (MI)
William Osler Health Centre (ON, Canada)
Winchester Hospital (MA)
Winn Army Community Hospital (GA)
Wishard Health Sciences (IN)
Womack Army Medical Center Department of Pathology (NC)
Womens and Childrens Hospital (LA)
York Hospital (PA)
Yukon-Kuskokwim Delta Regional Hospital (AK)

NOTES

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