CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

EP06

Evaluation of Linearity of Quantitative Measurement Procedures

This guideline provides information for characterizing the linearity interval of a measurement procedure, validating a linearity interval claim (to be performed by the manufacturer), and verifying an established linearity interval claim (to be performed by the end user).

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

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Evaluation of Linearity of Quantitative Measurement Procedures

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Abstract

Clinical and Laboratory Standards Institute guideline EP06—*Evaluation of Linearity of Quantitative Measurement Procedures* is intended to provide both manufacturers and users of quantitative measurement procedures with an economical and user-friendly method of validating and verifying the linearity interval. This guideline also can be used to determine the extent to which a quantitative measurement procedure meets medical requirements or the manufacturer's linearity interval claims.

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Foreword

A measurement procedure is **linear throughout a given interval** when, in that interval, the measured results "on average" (ie, abstracting from imprecision) are **proportional** to the measurand's true quantity values, meaning that the measurand results agree with the true values up to a constant multiplicative factor:

$$\text{Measured value} = k(\text{True value}) \quad (k > 0) \qquad (1)$$

A measurement procedure is **linear** (without additional qualification) when the procedure is linear throughout its stated analytical measuring interval. Thus, for example, in patient monitoring, when a measurand's true value doubles or decreases by 15% from one sample to the next, results obtained using a measurement procedure demonstrated to be linear can be expected (within limits determined largely by imprecision) to respectively double or decrease by 15%, although the procedure might exhibit systematic proportional bias relative to the measurand's true quantity values.

This characterization of linearity applies not only to measurement procedures that report results in concentration units (eg, nmol/L, ng/dL, µIU/mL), but also to those reporting enzyme activity, blood cell counts, etc. (For brevity, this guideline is written as if all such assays report in concentration units.) However, some tests reporting on a continuous scale, such as tests measuring specific patient (auto)antibodies, cannot be expected to show linear behavior for all patient samples. Moreover, the characterization is consistent with the use of "linear" and "nonlinear" terms in clinical chemistry as applied to conventional linearity-under-dilution studies. These studies typically involve preparing a spectrum of mixtures by combining a high-concentration sample with a measurand-free sample (or diluent), generating and averaging replicate measurement results for each mixture, and finally regressing these results vs the values expected from the high sample proportion (ie, relative volume) represented in each mixture. Success is demonstrated when, analytically and/or graphically, the paired values (ie, observed and expected results) all closely approximate a straight-line trajectory passing through the origin (0,0), making appropriate allowance for the measurement procedure's imprecision, the experiment's size, and clinically acceptable measurand- and concentration-specific deviations from the line.

The approach advocated in this edition of EP06, as well as previous editions, can be regarded as refinements of this conventional study with respect to design, analysis, and interpretation.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, EP06-A, published in 2003.

The first edition, EP06-P, published in October 1986, relied on fitting a straight line to measurements of five equally spaced samples, four replicates each, judging linearity by a goodness-of-fit test based on comparing dispersion around the regression line with the repeatability (ie, within-run imprecision) exhibited in the experiment. Unfortunately, this statistical test put measurement procedures with excellent repeatability at risk of inappropriately failing. Conversely, it might fail to identify nonlinearity in measurement procedures with very poor repeatability.

To rectify this shortcoming, the second edition, EP06-P2, published in December 2001, and the first approved guideline, EP06-A, published in April 2003, adopted a different and computationally more complex statistical test for linearity. EP06-A called for fitting not only first-order but also second- and third-order polynomials (ie, linear, quadratic, and cubic models) to the data, judging the measurement procedure to be linear if, by internal statistical criteria, the first-order fit is best. In effect, EP06-A asked whether the trajectory of experimental results had a shape more closely resembling a straight line rather than a parabolic or sigmoidal curve. Unfortunately, this method placed no restriction on the trajectory's orientation. EP06-A, unlike major publications cited therein, was not sufficiently clear that, with suitable

allowance for random error, the trajectory should be aligned with the origin. (Intuitively, for example, a measurement procedure exhibiting little or no decrease in measured results under progressive dilutions, such as so-called "analog" procedures for free thyroxine, is not considered linear even when the trajectory of results approximates a straight-line segment.)

This edition of EP06 builds on the previous editions, introducing several important refinements, including:

- The discussion of dilution schemes, designed to minimize errors in preparing the test panels, has been extended. There is no longer any suggestion that samples need to be equally spaced. This guideline encourages judicious interpolation of additional mixtures to improve coverage of concentration gaps between calibrators, as well as concentrations important for decision-making or monitoring.
- Like EP06-A, this edition emphasizes that suitable visualizations of the study data are important, and many examples are provided.
- Consistent with other CLSI method evaluation guidelines, this guideline calls for judging results in terms of the clinical acceptability of deviations (ie, deviations from linearity at each of the sample concentrations), as opposed to a global pass-or-fail assessment based solely on internal statistical criteria. This point of view makes this guideline's approach more relevant to clinical practice and more informative as to the location, magnitude, and significance of any deviations from linearity.
- Chapter 3 is devoted to validating linearity (intended for manufacturers and developers), and Chapter 4 covers verifying (ie, spot-checking) linearity (intended for end-user laboratories).
- Two study designs are discussed: one study design includes a high sample (whose concentration is known to exceed the procedure's analytical measuring interval) and a measurand-free sample. The other study design includes high and low samples with known concentrations or a known concentration ratio. These designs serve different purposes, have different limitations, and use somewhat different data analyses.
- Computationally, this edition's approach is simpler than that of EP06-A, insofar as fitting second- and third-order polynomials is no longer included for validating or verifying linearity (although developers might find such analysis informative). Conversely, weighted first-order regression analysis is recommended under appropriate circumstances to limit the risk of failure due to chance. Advice is provided on determining adequate sample-specific weights in the absence of a precision profile.
- The importance of stating a performance claim is emphasized.

NOTE: The content of this guideline is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

Linearity
Measured values
Measurement error
Proportionality
Weighted linear regression

Chapter 1

Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's content
- Standard precautions information
- Terminology information, including:
  - Terms and definitions used in the guideline
  - Abbreviations and acronyms used in the guideline

Evaluation of Linearity of Quantitative Measurement Procedures

1 Introduction

1.1 Scope

This guideline provides recommendations for designing, analyzing, and interpreting linearity studies for quantitative measurement procedures. This guideline is intended for manufacturers and developers seeking to validate the linearity of a measurement procedure throughout a stated concentration interval, especially the interval that includes the measurement procedure's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ). It is also intended for laboratorians who verify the linearity of a measurement procedure and for regulatory agencies responsible for overseeing *in vitro* diagnostic (IVD) manufacturers or end-user laboratories.

This guideline does not include information on linearity issues encountered during the measurement procedure development phase, such as efficiently identifying the widest possible interval for a linearity claim or selecting calibration points, although the experimental design and data analysis principles described herein can be of value during that phase.

Before the laboratory begins formal linearity verification studies, the measurement procedure's intended analytical measuring interval claim should already have been determined based on the results of linearity, precision, and other studies that have been evaluated using a clinically informed error budget for imprecision, bias, etc.

1.2 Background

EP06 is one of the CLSI method evaluation documents, which provide guidance on experimental evaluation of quantitative measurement procedures. These documents describe studies covering, eg, precision (see CLSI documents EP05[1] and EP15[2]), measurement procedure comparison and bias (see CLSI document EP09[3]), recovery (see CLSI document EP15[2]), and limits of quantitation (see CLSI document EP17[4]). EP06 is devoted to linearity studies.

The recommendations in this guideline differ depending on whether the study is intended to validate a measurement procedure's linearity or merely to verify it and also on whether fully commutable, measurand-free material is available for use as a diluent.

For verification, practical considerations may necessitate a smaller, less rigorous study than would be required to validate performance claims for regulatory purposes. For example, compared with validation, verification may involve fewer samples (ie, mixtures, dilutions), fewer replicates, and often, for at least two reasons, sample concentrations that span only a large segment of the measurement procedure's stated analytical measuring interval. First, owing to software constraints, end users might not be able to generate explicit numerical results for samples with concentrations beyond the upper and/or lower limits of the procedure's analytical measuring interval. Moreover, owing to the procedure's inherent imprecision, laboratories might not be able to generate consistent results for samples very close to (but within) these limits. Second, to accommodate multiple commercial measurement procedures for a given measurand that differ in their stated analytical measuring intervals, third-party providers of samples for linearity (or calibration) verification studies sometimes restrict the samples' concentration span for that measurand to an interval deemed medically essential for any such procedure.