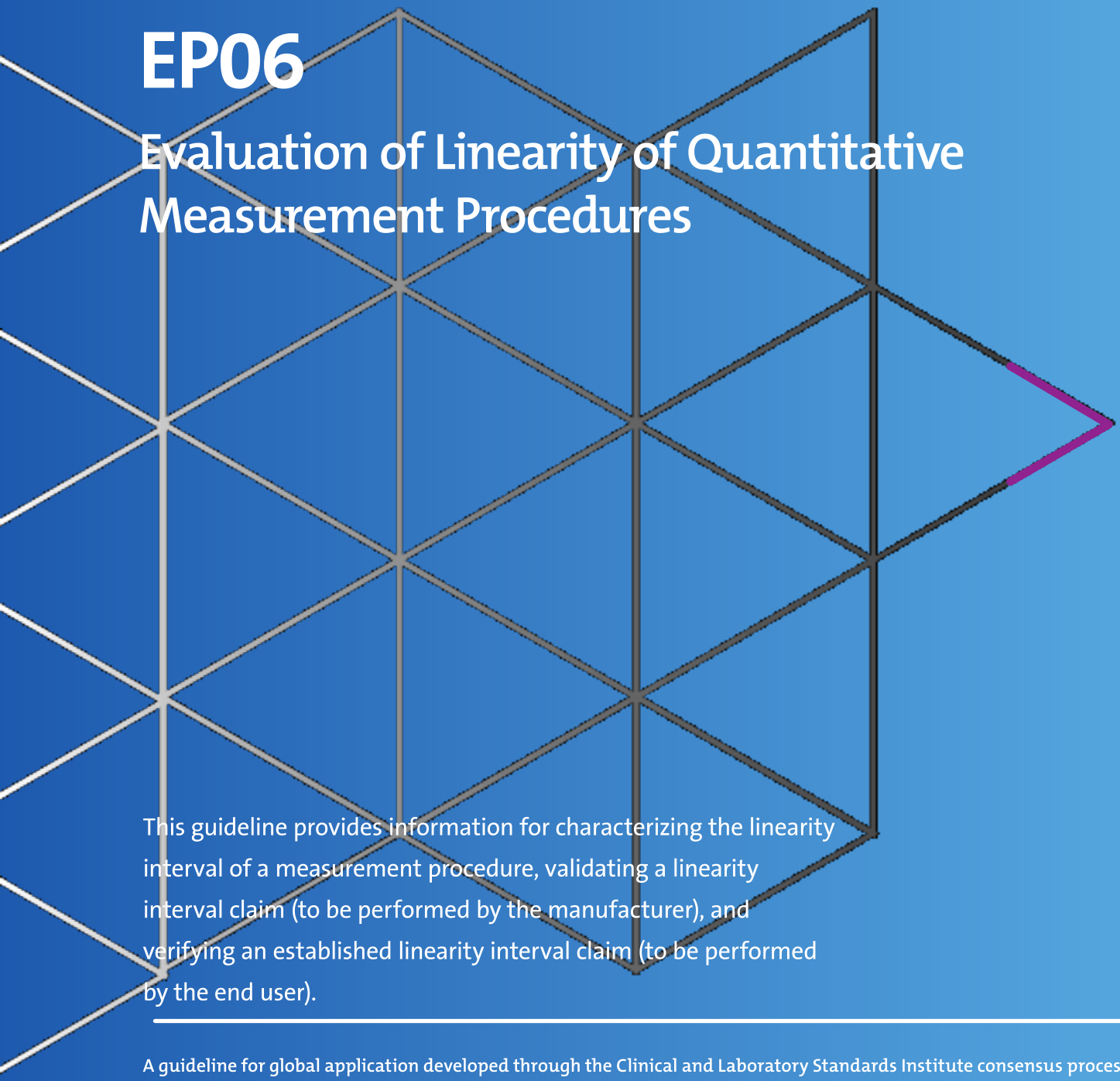
CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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

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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:

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

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 cognate 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 puts 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