CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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EP06-A

Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

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.

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

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Abstract

CLSI document EP06-A—*Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline* is intended to provide both manufacturers and users of quantitative analytical methods with an economical and user-friendly method of establishing or verifying the linear range. This guideline also can be used to demonstrate the extent to which a quantitative analytical method meets clinical requirements or manufacturer's linear range claims.

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Foreword

A quantitative analytical method is linear when there exists a mathematically verified straight-line relationship between the observed values and the true concentrations or activities of the analyte. The property of being linear is important for analytical and clinical laboratory methods. The linear relationship is valuable because it represents the simplest mathematical relationship and allows for simple and easy interpolation of results.

It has been argued that linearity is not necessary for such methods as competitive immunoassays, because the relationship between the response of the system and analyte concentration is inherently nonlinear. However, the mathematical relationship between the response and analyte concentrations should be sufficiently well defined to allow the selection of a suitable transformation of the dose-response curve to linear form. Furthermore, for an analytical method, one cannot interpolate between points unless one knows the results are linear.

The reason for ensuring linearity of analytical methods is important on clinical grounds. Clinicians know that the relationship between an analyte and the pathophysiologic process is usually nonlinear, but they expect that the results reported to them by the laboratory include a linear relationship between the result and the true concentration, count, or activity recovered. For example, if the true amount of an analyte in a sample were to double, clinicians expect that would be reflected in a doubling of the measured value.

This Revision

The approach outlined within the approved version of EP06 represents a significant change in statistical approach from the previous version.

The first edition (EP6-P) used the statistical “Lack of Fit” test (LoF) as the sole basis for determining linearity. With this protocol, five equally spaced concentrations were analyzed with four replicates at each level. A regression line was fit to the points, and two variance estimates were produced – the pooled variance between replicates and the variance of the five means around the regression line. The ratio of these variances was the basis for the LoF test. One problem with this approach is that very precise systems have small variance between replicates, and in the ratio LoF test, very small deviations of the means were seen as statistically significant – or nonlinear. It was also possible – though less frequent – that extremely large variance between replicates could lead to a failure to detect clinically important nonlinearity.

This polynomial method (EP06-A) is conceptually very similar to the original LoF test. In both procedures, there are two alternative statistical models (linear and nonlinear); and they are assessed relative to which is most likely to be true. However, with the polynomial approach, there is a specific parametric model for the nonlinear alternative, to better identify specific nonlinear conditions, and pure repeatability precision is included in a positive way. Better precision leads to better decisions and poor precision is screened out before linearity decisions are made. This approved statistical approach:

- estimates the magnitude of nonlinearity at every level;
- controls for unacceptable repeatability in the assessment of nonlinearity;
- provides a testable statistical model;
- can be programmed easily with widely available software.

Another key concept from the previous version has been retained, i.e., the necessity to plot the data as a first step in the evaluation of linearity. The visual examination will help the user decide the extent to which the statistical assessment can be useful for determining whether there is significant nonlinearity, and the magnitude of the nonlinearity relative to predetermined goals.

A Note on Terminology

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

In keeping with NCCLS's commitment to align terminology with that of ISO, the following terms are used in EP06: *Trueness* is used in this document when referring to the closeness of the agreement between the average value from a large series of measurements and to a true value of a measurand; *Repeatability* has replaced the term *Within-run precision* where appropriate, when describing the closeness of agreement between results of successive measurements of the same measurand carried out under the same conditions of measurement; *Measurement procedure* has replaced the term *Analytical method* for a set of operations, used in the performance of particular measurements according to a given method; *Measuring range* has replaced *Reportable range* when referring to a set of values of measurands for which the error of a measuring instrument is intended to lie within specified limits; *Measurement error/Error of measurement* is used instead of *Total error* to describe the result of a measurement minus a true value of the measurand.

At this time, the working group has chosen not to replace A*nalyte* with *Measurand,(i.e.,* particular quantity subject to measurement) due to user nonfamiliarity and for the sake of the practicability of the guideline.

Users of EP06-A should understand, however, that the fundamental meanings of the terms are very similar, and to facilitate understanding, the terms are defined along with explanatory notes in the guideline's Definitions section.

All terms and definitions will be reviewed again for consistency with international use, and revised appropriately during the next scheduled revision of this document.

Standard Precautions

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

Key Words

Allowable difference, allowable error, linearity, matrix effects, measurement error, total error, uncertainty

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1 Scope

This document presents a method to establish, verify and/or demonstrate the linear range of a quantitative measurement procedure. These methods do not identify the causes of significant nonlinearity. The method employs increasing numbers of samples for more definitive examinations of linearity. Therefore, if a failed demonstration is evaluated and it is determined that the experiment needs to be repeated, it can be done with more replicates or with fewer levels to cover a smaller range.

- This protocol is to assess linearity, isolated as much as possible from conditions of precision and trueness. It is understood that poor precision will hinder an effective assessment of linearity, so a check for poor repeatability is included.

- These experiments should use samples with a matrix appropriate to the specimens being analyzed (serum, plasma, urine, etc.)

- This protocol requires laboratories to set goals for nonlinear error. It provides basic concepts for setting such goals, but does not recommend any specific protocol.

2 Introduction

2.1 Purpose

The purpose of this guideline is to describe a statistical process for determining the linearity of a quantitative measurement procedure. This primary objective is to determine the concentration(s) where a method is not linear and the extent of the nonlinearity at that level. This guideline emphasizes the necessity that each user establishes his or her requirements for linearity, or the allowable error due to nonlinearity. It also places less importance on global tests for linearity across the tested range (such as the LoF test). Global tests merely indicate that statistically significant nonlinearity exists; they do not show where that nonlinearity is, nor do they show the magnitude of the error. Linearity tests can be helpful to assess bias, which is a component of measurement error, but nonlinearity is not the only component of bias.

Users should have an understanding of their needs for measurement error, bias, random error (or imprecision), and nonspecificity (or interferences). From these they can derive a goal for linearity. In this context, NCCLS document EP06-A is a part of a series of documents that guide users through the process of method evaluation. Also see the most recent version of NCCLS document EP21—*Total Analytical Error for Clinical Laboratory Methods.*

This document is meant to cover a broad range of situations, such as establishing the linear range of a method, which requires testing across a wide range of concentrations, then progressively narrowing into a range of acceptable linearity. It is also intended to cover situations where the linear range has been determined elsewhere (e.g., by the manufacturer), but the user wishes to verify that range in their laboratory. The same procedure is used for all scenarios, but with different numbers of concentration levels and different numbers of replicates.