

Evaluation of Matrix Effects; Approved Guideline—Second Edition

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.

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



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ISO/TC 212 standards, and ISO/TC 76 standards*

Clinical and Laboratory Standards Institute

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Evaluation of Matrix Effects; Approved Guideline—Second Edition

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Abstract

Clinical and Laboratory Standards Institute (CLSI) document EP14-A2—*Evaluation of Matrix Effects; Approved Guideline—Second Edition* was developed for manufacturers, regulators, and providers of proficiency testing or external quality assessment programs, although it will be useful to clinical laboratories as well. The document will help users to determine whether matrix effects are the source of unexpected results that are sometimes observed with processed samples when two measurement procedures are compared; to identify and quantify the magnitude of the effects; and to ensure that laboratory performance is evaluated fairly if matrix effects are present. The suggested protocols were developed using patient specimens as the standard of comparison. A list of definitions is included.

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Foreword

The presence of matrix effects in measurement procedures used in the clinical laboratory has been a source of serious concern for many years. Although in the literature, there are many references to the apparent incompatibility of fluids and measurement procedures, when this work was first proposed there were no generally accepted guidelines that demonstrate how to identify and quantify the magnitude of the bias caused by matrix effects. Because these effects are commonly observed in external quality assessment (EQA) schemes or proficiency testing (PT) results, protocols are needed to determine the presence or absence of these effects. Only then can the laboratorian assess whether the observed effect(s) will have an impact on patient care.

Determining the presence or absence of matrix effects allows users, manufacturers, and those responsible for evaluating EQA and PT data to distinguish between a true malfunction of the measurement procedure and incompatibility between the procedure and the material being tested.¹ The real difference is that measurement procedure malfunctions affect patient care, while matrix effects limit how the procedure can be evaluated and monitored. When matrix effects are present with procedure calibrators, calibrator values should be adjusted so that reported patient results are not affected. In fact, this has become standard practice among manufacturers.^{2,3}

The Working Group on Matrix Effects was faced with a practical dilemma of definition. If a difference in results between measurement procedures is observed with processed samples using these protocols, an interfering substance might be present. However, its source is not known in this early evaluation stage; it could be caused by a specific substance(s) or by the matrix—the milieu of the sample that differs from the specimens for which the procedure was designed. It could also be caused by differences between the analyte of interest and the actual measurand (the quantity that is intended to be measured). We decided for the purposes of this document to use the broadest interpretation; that is, this procedure is an effective way to identify whether an unexpected difference in results is observed in processed samples, and we direct the user to CLSI/NCCLS document EP7—*Interference Testing in Clinical Chemistry* to test the source of the bias and quantify its magnitude in terms of the analyte and interfering substances.

The working group believes these protocols and the supporting information will be most useful to manufacturers and providers of external evaluation programs. Our objective is to provide ways to identify the presence of matrix effects so that improvements in method specificity and fluid compatibility (controls and calibrators) can be made, and to provide government regulators with a mechanism that can be used to distinguish between laboratories that are doing acceptable work from those that need improvement (based on the results of EQA/PT). The working group anticipates that this guideline will be helpful when differences in results between measurement procedures are observed with control or proficiency test materials that might affect an understanding of method performance.

Trueness, traceability, and commutability are of current interest, collectively and independently, to help achieve consistent and accurate clinical measurements for patient benefit, regardless of where a measurement procedure is performed. The protocols in EP14 have been suggested as useful for identifying commutable materials.⁴ Although we do see the potential for such use, we are cautious in recommending it without modification. Procedures to provide high assurance that a material is intended as a “universal” calibrator must be assessed with greater rigor (more fresh patient specimens, more reagent and calibrator lots, more runs) than these procedures provide. This could be the objective of another guideline or as an addendum to future editions of EP14. Another method has been proposed recently to demonstrate commutability of materials, with the use of interlaboratory assessment schemes in which a number of measurement procedures are used routinely.⁵

The general rationale used to develop each protocol was that clinical laboratory procedures are designed and developed to work optimally with patient specimens. Characteristics of manufactured control or calibrator materials that deviate significantly from the way patient specimens behave in specific

procedures, with whatever response characteristics are used for measurement, can be called “matrix effects” because the source of the difference has not been identified. Pragmatically, for this document, an observed difference of unknown source is called a “matrix effect,” while a difference due to an identifiable substance or physical characteristic is an “interference” (see Appendix A), and the user is referred to CLSI/NCCLS document EP7—*Interference Testing in Clinical Chemistry*. Definitions are streamlined to account for known and unknown interferences.

The limitations of these protocols include (but are not limited to) the following:

- Subtle analytical differences that occur with consistency between different procedures for measuring a given analyte may not be easily detectable. These protocols may not be sufficiently powerful to detect or identify the presence of these differences. (Protocols described in Sections 6.3(6) or 6.4(2) could be helpful.)
- No attempt is made to determine the clinical or regulatory significance of the magnitude of difference or bias between measurement procedures. However, the magnitude of the bias or difference might be used to compare to independently derived clinical or regulatory (e.g., PT) limits.
- These protocols cannot determine which of the two procedures is more specific for measuring or for accurately detecting an analyte in a particular fluid.
- These protocols might not be usable within all disciplines of clinical analysis.

Lastly, elimination of matrix effects requires either an improvement in the analytical specificity of procedures or in the materials used for quality control, calibration, and/or external assessment. The clinical laboratory testing community should not lose sight of the fact that, in a perfect world, there would be no “matrix effect.” In such a world, every routine method would have sufficient analytical specificity to produce accurate results with any fluid or material. This lack of analytical specificity, however, is the reason this guideline is needed.

A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. 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, 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, CLSI recognizes that harmonization of terms facilitates the global application of standards and deserves immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In order to align the usage of terminology in this document with that of ISO, the following terms are used in EP14-A2:

The term *trueness* has replaced the term *accuracy* when referring to the closeness of agreement between the *average value* obtained from a large series of test results and an accepted reference value. *Accuracy*, in its metrological sense, refers to the closeness of the agreement between the result of a *single measurement* and a true value of a measurand, thus comprising both random and systematic effects.

The term *measurement procedure* has replaced the terms *method*, *analytical method*, and *analytical system* for a set of operations used in the performance of particular measurements according to a given method. However, for ease in writing the document, “comparative method” and “evaluated method”

have been retained, and are understood to represent the two measurement procedures under study with this protocol.

The terms *specimen* and *sample* are both used in this document, with *specimen* reserved for actual patient materials, and *sample* reserved for processed materials (e.g., PT samples, reference materials).

The terms *measurand* and *analyte* are used appropriately in this document, with *analyte* used to represent the particular component of interest to the patient, and the term *measurand* used to describe the specific quantity that is measured by a particular measurement procedure (i.e., the measurand describes what is actually causing the result of the measurement). This important difference can be subtle since it can be due to the detection of different measurands in the procedures being compared.

To facilitate understanding, the terms are defined in the Definitions section (see Section 4). All terms and definitions will be reviewed again for consistency with international use, and revised appropriately during the next scheduled revision of this document.

Key Words

Analytical interference, bias, matrix, matrix effect, physicochemical interference

Evaluation of Matrix Effects; Approved Guideline—Second Edition

1 Scope

This guideline is intended for diagnostic test manufacturers, external quality control and proficiency testing providers, and regulatory agencies. Although clinical laboratory use will probably be limited because of the complexity of the calculations, the observations and conclusions should be useful to all professionals. The guideline provides protocols that evaluate matrix effects in processed samples that are used as standards, calibrators, controls, and EQA/PT materials.

EP14 will assist in the education of clinical laboratorians, regulators, diagnostic manufacturers, and the public about the impact of matrix effects on the assessment of the quality of laboratory performance. For example, readers are warned that matrix effects, caused by the interaction of processed material and the measurement procedure, may suggest that erroneous results are being generated when in fact the results are acceptable. Conversely, “acceptable” control results may also give a false sense of confidence that procedures are performing adequately. Terms and concepts used to report these and related issues are defined within this document.

This guideline can be used by laboratorians performing quantitative tests for a wide variety of analytes across various disciplines. The testing protocols attempt to accommodate situations where reference methods do not exist.

The protocols help laboratorians distinguish between effects caused by measurement procedure malfunctions and those caused by use of processed samples. However, the protocols do not describe approaches that specifically establish the exact mechanism of the matrix effect(s).

By following the protocols, manufacturers and EQA/PT providers should be able to provide some documentation to government or accrediting agencies on matrix effects to help avoid false conclusions about the adequacy of patient testing.

2 Introduction

The interest in trueness (earlier commonly described as “accuracy”) of testing in biological fluids has grown among the medical and laboratory professional community, as well as with the public. Regulations and standards are in place that are meant to enhance the trueness of the testing process. There is renewed emphasis on the use of external quality assessment schemes and proficiency testing to evaluate and monitor the trueness of testing in clinical, reference, and physician’s office laboratories.

Current scientific data suggest that such use of EQA/PT results is not always feasible because of matrix effects, which exist with many external control materials. These processed materials (including quality control and calibrating materials) sometimes do not behave like the fresh specimens routinely analyzed in the laboratory. Biases not generally seen with fresh biological fluids are frequently seen with EQA/PT, control, and calibrator materials. Because of these matrix effects, evaluating laboratory performance for trueness of testing using EQA/PT can lead to inaccurate conclusions and, potentially, inappropriate regulatory sanctions.

Matrix effect phenomena involve the interplay of four major components in analytical testing: instrument design; reagent formulation; measurement principle; and control, calibrator, and EQA/PT material composition and processing technique. Within each of these categories are factors that contribute to the magnitude and direction (positive or negative) of the bias. The interactions that cause these matrix effects are complex and differ by discipline (e.g., chemistry, hematology) and by the nature of the materials used

to calibrate and monitor performance of each method. For example, the performance characteristics of a cellular suspension would be expected to differ from those of a protein-free filtrate.

Research is needed to characterize these interfering factors so that instruments, reagents, and fluids can be designed to minimize them. Until then, standardization of methods, as well as assessing or monitoring the trueness of laboratory testing, will be difficult.

This document is complementary to CLSI/NCCLS document EP7—*Interference Testing in Clinical Chemistry*. They are similar in that both provide protocols to help identify sources of error that can affect patient care and/or assess the suitability of a method. They are distinguishable in the following areas:

- EP14 focuses primarily on the difference between processed samples and patient specimens, while EP7 concentrates on how specific substances or conditions (e.g., the presence of an interfering substance or a change in viscosity) alter results in patient specimens.
- To evaluate the effect of interferences, EP14 compares performance of processed samples to a population of patient specimens, whereas EP7 uses criteria based on the precision of the measurement procedure and the intraindividual variability of the measurand in the presence of increasing amounts of the interfering substance.
- The criteria used to determine if an effect is present are based on the dispersion of results from the patient specimens about the line of best fit in EP14, whereas EP7 uses the uncertainty of replicate measurements of a series of related pools that contain differing, known amounts of the substance (or change in condition) being investigated.
- EP14 compares the bias of processed samples to that of patient specimens, while the objective of EP7 is to quantify the observed difference as a function of the concentration of the interfering substance (or other characteristic) at specified concentrations of analyte.

3 Standard Precautions

Because it is often impossible to know what 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 infectious agents and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard 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;17(1):53-80 and *MMWR* 1988;37:377-388). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of CLSI/NCCLS document M29—*Protection of Laboratory Workers From Occupationally Acquired Infections*.

The identification of matrix effects requires the use of controls, calibrators (standards), and materials used in external quality assessment schemes, as well as patient specimens, because it is necessary to compare results obtained with these materials. Most control and calibration materials have been treated to denature HIV and HBV, but they should still be handled with the same precautions as patient specimens. Extensive pipetting of these materials may be necessary for making dilutions. These samples should never be pipetted by mouth. Pipetting aids are available for every task. Bulbs or other suitable suction devices must always be used with pipettes.

Controls, calibration materials, or diluents may contain azide, which is toxic. Azide may also form explosive compounds if it comes in contact with copper and lead plumbing. Products that contain azide should be flushed with excess water upon disposal down drains.

Materials for microbiological analysis should be handled in strict accord with the accepted techniques used to prevent the spread of the suspected organisms. Isolation hoods and sterile techniques should be used when indicated. Care should be taken to avoid forming aerosols. Because controls and standards of bacterial and viral assays may contain viable organisms, these should be handled appropriately.

4 Definitions

Definitions are provided as they apply to this document. Some differ from other CLSI/NCCLS documents because of the pragmatic requirements of these protocols. The use of a hierarchical approach to the source of observed differences (interferences) is illustrated in Appendix A, which should be used with definitions listed below.

Accuracy (of measurement) – Closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93)⁶; See **Measurand**, below.

Analyte – Component represented in the name of a measurable quantity (ISO 17511)⁷; **NOTES:** a) In the type of quantity “mass of protein in 24-hour urine,” “protein” is the analyte. In “amount of substance of glucose in plasma,” “glucose” is the analyte (ISO 17511)⁷; b) The analyte is the particular component of interest to the patient.

Analytical interference – The effect of a substance, either identified or unidentified, that causes a difference in the measured concentration or activity from the true value⁸; **NOTES:** a) The difference is systematically related to the concentration of the analytical interfering⁹; b) See also **Physicochemical interference**.

Bias – Difference between the expectation of the test results and an accepted reference value (ISO 3534-1).¹⁰

Calibration – Set of operations that establishes, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards (VIM93)⁶; **NOTE:** According to the U.S. Code of Federal Regulations, calibration is the process of testing and adjustment of an instrument, kit, or test system, to provide a known relationship between the measurement response and the value of the substance being measured by the test procedure (42 CFR 493.1217).¹¹

Commutability (of a material) – Ability of a material to yield the same numerical relationships between results of measurements by a given set of measurement procedures, purporting to measure the same quantity, as those between the expectations of the relationships obtained when the same procedures are applied to other relevant types of material (EN 12287).¹²

Comparative method – The measurement procedure used as the basis for comparing two different measurement procedures (e.g., in the evaluation of matrix effects); **NOTE:** The more specific this procedure is, the better the conclusion with regard to the source of the observed interference.

Evaluated method – That measurement procedure for general clinical use that is being evaluated for a possible matrix effect.

External quality assessment/Proficiency testing (EQA/PT) – A program in which multiple samples are periodically sent to members of a group of laboratories for analysis and/or identification; in which each laboratory's results are compared with those of other laboratories in the group and/or with an assigned value.

Imprecision – Dispersion of independent results of measurements obtained under specified conditions; **NOTE:** It is expressed numerically as **Standard deviation** or **Coefficient of variation**.

Isoform – One of several forms of a single protein that have the same antigenic structure but that differ in minor amino acid content and/or steric structure.

Matrix – All components of a material system, except the analyte (modified from EN 12286).

Matrix effect – Influence of a property of the sample, other than the analyte, on the measurement, and thereby on the value of the measurable quantity (EN 12287)¹²; **NOTE:** The physicochemical effect(s) (e.g., interference) of the matrix on the measurement procedure's ability to accurately measure an analyte.

Measurand – Particular quantity subject to measurement (VIM93)⁶; **NOTES:** a) This term and definition encompass all quantities, while the commonly used term **Analyte** refers to a tangible entity subject to measurement (i.e., the measurand describes what is causing the result of the measurement; and the analyte describes the particular component of interest to the patient); b) See **Analyte**, above.

Measurement method – Logical sequence of operations, described generically, used in the performance of measurements (VIM93).⁶

Measurement procedure – Set of operations, described specifically, used in the performance of particular measurements according to a given method (VIM93).⁶

Observed response – The measured physical or chemical parameter used to identify or quantify an analyte in comparison to an appropriate calibration system; **NOTE:** The observed response may be used by a system's internal processor and, therefore, the value is often not available to the testing personnel; examples include absorbance units, radioactive counts, and millivolt readings.

Physicochemical interference – An environmental or structural difference from that of the patient specimens that causes a difference between the population mean of the test results and an accepted reference value due to a change in the measured physical chemical property⁸; **NOTE:** This is what has commonly been referred to as "matrix effect"; examples include the effect of different protein matrices on bilirubin spectra or the impact of proteins and lipids on the measurement of electrolytes in plasma in direct ion-selective electrode systems. See also **Analytical interference**.

Processed sample – For the purposes of this document, a sample that is prepared to be used to mimic patient specimens; **NOTE:** a) It is considered a processed sample if it has been modified in any way that causes it to be different from fresh patient specimens, for example, freezing, lyophilization, adding nonendogenous substances, stabilizers, etc.; b) For EP14, these are the materials being evaluated for matrix effects.

Residual – The difference between a given data point and its predicted value.

Sample – One or more parts taken from a system, and intended to provide information on the system, often to serve as a basis for decision on the system or its production (ISO 15189)¹³; **NOTE:** For example, a volume of serum taken from a larger volume of serum (ISO 15189).¹³

Specificity – The ability of a test or procedure to correctly identify or quantify an entity in the presence of interfering phenomena/influence quantities; **NOTES:** a) In quantitative testing, the ability of a measurement procedure to determine only the component it purports to measure or the extent to which the assay responds only to all subsets of a specified analyte and not to other substances present in the sample; b) For qualitative or semiquantitative tests, the method's ability to obtain negative results in concordance with negative results obtained by the reference method.

Specimen – Biological material which is obtained in order to detect or to measure one or more quantities.

Trueness – Closeness of agreement between the average value obtained from a large series of test results and an accepted reference value (ISO 3534-1).¹⁰

5 Principle of Evaluation

The evaluation of a matrix effect is based on the principle that the relationship between an observed response and the actual activity or concentration is often dependent upon the environmental conditions (e.g., temperature or matrix) at the time of measurement.¹⁴ Because few, if any, measuring techniques are completely specific, the observed relationship between any two measurement procedures will depend on the choice of the samples selected for comparison.¹⁴ For clinical laboratory analysis, measurement procedures are designed to measure the concentration or activity in patient specimens, and a representative set of these specimens is used as the standard of comparison.

The magnitude of the matrix effect is evaluated by comparison to the “scatter” of results from the two measurement procedures being compared using a representative sample of patient specimens. The more heterogeneous the specimens, in terms of the interfering substance or difference in matrix, the larger the scatter expected in the data.

The magnitude of the difference in the processed sample(s) is compared to the resultant scatter of the patient specimens. This residual scatter represents the uncertainty of measurement of the evaluated method due to two factors: imprecision and nonspecificity. (The regression techniques used in these protocols use the assumption that there is no error in the comparative method represented on the x-axis.) The contribution of imprecision is reduced by replicate measurements in both the evaluated and the comparative procedures; therefore in these analyses, the primary contributor to scatter is the inherent interferences due to substances that are known or unknown (here called a “matrix effect”). The range of the scatter is represented by the prediction interval, which estimates the nonspecificity of the evaluated method for all patient specimens that would be tested. It is then possible to assert with reasonable probability whether the processed sample can be used to represent the set of patient specimens for the analyte being measured¹⁵; if the processed sample(s) result(s) is outside of the prediction interval, a matrix effect is present.

Additionally, if a series of processed samples are related (as is often the case in an EQA/PT event), such as being prepared from admixtures of common pools, regressing the results of these samples and comparing the line of best fit to the prediction interval can be used as a means of evaluation. This technique is especially helpful if the matrix effect is within the prediction interval, but is consistent or shows a relationship across all related processed samples.

Any conclusions from the study are limited to the specific variables of the processed samples (e.g., manufactured batch, sources of analytes used to supplement the sample, types of stabilizers that might be present). An additional limitation is that each method can be quantifying different measurands that have been associated with the same clinical condition of interest. Follow-up studies might be required to determine the source(s) of the observed differences.

6 Protocols

6.1 Materials

The following materials are needed for these protocols:

- evaluated method reagents, calibrators, and instrument system.
- comparative method reagents, calibrators, and instrument system. Use a method expected to show little or no matrix effect with processed calibrator or control samples. In order of preference, the comparative methods should fit the following descriptions, for example:
 - a National Reference System (NRSCL) primary reference measurement procedure (e.g., isotope dilution-mass spectrometry method for cholesterol);
 - an NRSCL secondary reference measurement procedure (e.g., the Abell-Kendall method for cholesterol);
 - an NRSCL-approved designated comparative method;
 - a commonly used method for the particular analyte in question.

NOTE: Although ideally, the comparative method should be free of matrix effects, this cannot be an absolute requirement. For practical reasons, a frequently used commercially available measurement procedure may be selected as the comparative method. When matrix effects are detected, however, the information obtained from these protocols will merely indicate that patient specimens and processed samples do not yield comparable results when used to measure a particular analyte with both methods. These protocols will not identify whether the evaluated method or the comparative method has better specificity.

- processed samples (e.g., reference materials, calibrators, control samples being studied).
- twenty fresh patient specimens with analyte concentrations or activities that are approximately evenly distributed over the concentration range of the processed samples of interest. Select patient specimens that are typically used for analysis (e.g., from both healthy and ill patients), and avoid those that are considered inappropriate for analysis due to the presence of known interferences. Frozen specimens may be included if freezing does not affect the measurements of either method.

6.2 Procedure

- (1) Prepare the processed samples as directed.
- (2) Using the evaluated method, analyze as a single analytical batch the 20 fresh patient specimens, with processed samples randomly interspersed between the fresh patient specimens. Repeat this process twice (sequential batches on a single day are preferable to eliminate the potential of shifts or drifts that can confound the data), preferably with separate calibrations. This yields three analytical results for each of the 20 patient specimens and the processed samples (see Appendix B). Perform a check for outliers, as recommended in CLSI/NCCLS document EP9—*Method Comparison and Bias Estimation Using Patient Samples*.
- (3) Using the comparative method, analyze (as a single analytical run or batch) the same 20 fresh patient specimens, with the same processed samples randomly interspersed between patient

specimens. Analyze the fresh specimens and processed samples at the same time as the evaluated method analyses. Repeat this process twice, preferably with separate calibrations. Perform an outlier check as recommended in CLSI/NCCLS document EP9—*Method Comparison and Bias Estimation Using Patient Samples*. If simultaneous analysis is not possible, information should be available to demonstrate that the comparative method results are not changed by the storage conditions used for the fresh patient specimens and for the processed samples.

- (4) Freeze (preferably at -70 °C) the 20 patient specimens and processed samples for future analysis. If any questions arise during or after data analysis, the specimens may be reanalyzed using another comparative method (e.g., an NRSL definitive or reference measurement procedure). Keep in mind that freezing may introduce a matrix effect by altering binding proteins, enzyme conformation, etc.

6.3 Data Analysis

As often occurs in statistical analysis, the user is asked to judge the utility and appropriateness of the statistical test for each data set. In these analyses, linearity, heteroscedasticity, and each method's imprecision could affect interpretation of results. Use of incorrect assumptions will result in more difficulty in identifying the presence of a matrix effect; the prediction interval from the patient specimen set will be wider. We remind the user to keep in mind the intended purposes of each study. Standard statistical textbooks can be referenced.

- (1) Plot the means of replicates of the 20 fresh patient specimens and the processed sample(s) (using different symbols) with the evaluated method results on the y axis and the comparative method on the x axis.
- (2) Examine the distribution of the means of results from the fresh patient specimens obtained using the evaluated and comparative methods and verify the following prerequisites:
 - Linear Regression Analysis
 - The appearance of a linear relationship between the evaluated method and the comparative method results from patients' specimens without any noticeable curvature.
 - The scatter in the y-direction around the regression line appears constant across the concentration range examined.
 - Check the appropriateness of the data for regression analysis (refer to the most current edition of CLSI/NCCLS documents EP6—*Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach* and EP9—*Method Comparison and Bias Estimation Using Patient Samples*).

Then perform linear regression analysis using the means of the evaluated method results (from patient specimens) as the y value and the means of the comparative method results (from patient specimens) as the x value (see Appendix C, Example 1).

- Polynomial Regression Analysis

The linearity of the evaluated method results and the comparative method results for the fresh patient specimens should be checked using procedures outlined in CLSI/NCCLS document EP6—*Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*, using the mean values of replicate testing from each method and including calculation of the best fitting polynomial regression model. This will provide the smallest prediction intervals (see below) and

optimal ability to detect significant matrix effects. As an example, if the best fitted polynomial is a second-order polynomial with the mean of the values of the evaluated method results as the y values and the means of the comparative method results as the x values (see Appendix C, Example 2), then:

$$y = a_0 + a_1x + a_2x^2 \quad (1)$$

If the a_2 coefficient in the second-order polynomial regression model is statistically different from zero (i.e., $p < 0.05$ determined by the t -test), use the second-order polynomial. If a_2 is not statistically different from zero (i.e., $p > 0.05$), use the first-order polynomial.

NOTE: Caution is advised to declare a method nonlinear based on 20 patient specimens, depending upon the distribution of their concentrations. Adding specimens to obtain a wider spread of x -values might be advisable. If the data are fitted to a first-order polynomial by regression analysis (rather than higher-order polynomials) for methods that are not linear, it will be more difficult to determine whether or not a matrix effect is present. However, since linear regression analysis is usually more convenient, and if the matrix effects are large, starting with linear regression might satisfy the needs of the user. Additionally, unless the characteristics of the comparative method are well understood, as with reference measurement procedures, the user will not be able to determine from these experiments alone, whether the source of nonlinear response is from the evaluated method, the comparative method, or both methods.

NOTE: If the linear regression model is used when the relationship between the two methods is, in fact, curvilinear, the calculated prediction interval about the line will be broader. Therefore, it will be more difficult to identify a real difference (a matrix effect) between processed samples and patient specimens.

- (3) If the scatter of patient specimen results around the regression line seems to increase in proportion to the measurand concentration, rather than being constant across the concentration range (i.e., standard deviation divided by concentration is constant, rather than standard deviation itself being constant across analyte concentration), perform a $\log_{10}$ transformation of the means of the evaluated method and comparative method results or, alternatively, plot results on log/log graph paper. Proceed through steps 1, 2, and 3 above; however, plot and perform linear or second-order polynomial regression analysis on the $\log_{10}$ -transformed means, instead of the means themselves. To effectively estimate the magnitude of scatter around the regression line, the variance of that scatter, or a transformation of the variance, must be constant.
- (4) Using the formula shown below, compute the two-tailed 95% prediction interval for the mean of the fresh patient specimen y value at a given x value (the mean of the replicate measurements) about the least-square linear regression line, the second-order polynomial regression line, or the $\log_{10}$ -transformed (for y variable) regression line.

$$\bar{Y}_{\text{pred}} \pm t(0.975, n - g) S_{y \cdot x} \left[1 + \frac{1}{n} + \frac{(\bar{X}_i - \bar{X})^2}{\sum (\bar{X}_i - \bar{X})^2} \right]^{1/2} \quad (2)$$

where:

$\bar{Y}_{\text{pred}}$ = the predicted value of y at X_i based on an estimated regression curve;

n = the number of fresh patient specimens (not the total number of replicates);

g = 2 for linear regression and $g = 3$ for second order (quadratic) regression;

$S_{y \cdot x}$ = the standard error of regression =

$$[\sum (Y_{\text{pred}} - \bar{Y}_i)^2 / (n - g)]^{1/2};$$

$\bar{X}_i$ = i th value on the x axis (comparative method mean);

$\bar{Y}_i$ = i th value on the y axis (evaluated method mean); and

$\bar{\bar{X}}$ = the overall grand mean of the comparative method means.

Compare each individual processed sample's mean y result to the statistically defined limits (95% prediction interval) derived from all patient specimen data points using the equation. The user is reminded that if large differences exist in specificity of the methods used, poor correlation (large prediction interval) will result, making this procedure less or not effective. See examples in Appendix C.

- (5) Alternatively, the prediction interval for the y values can be calculated for the corresponding series of x values and plotted on the graph along with the regression line for easy evaluation. Compare the magnitude of the processed sample deviation from the regression line (fresh patient specimens) to the 95% prediction intervals on the graph as illustrated in Appendix C. A matrix effect is present if the result of the processed sample(s) lies outside the prediction interval (see Appendix C, Example 1). If the processed sample result is within the prediction interval, a matrix effect is probably absent (see Appendix C, Example 2). However, if a persistent bias is observed among a set or group of processed samples and some or all of the biases are within the prediction interval, a matrix effect cannot be ruled out. If these sample sets are known to be related, such as admixtures of the same master pools, use the procedure described in Section 6.4(2) to aid in evaluation. The "allowable" or "acceptable" limit of the residual at any concentration would be evaluated against independent criteria.

NOTE: Caution is advised because PT limits published in the CLIA regulations are for single measurements of controls, while these protocols recommend using the mean value of triplicate assays. Therefore, a single measurement, performed for PT purposes, is not of equivalent experimental design, and therefore is not recommended to evaluate whether or not a matrix effect is present.

NOTE: Matrix effects that are statistically significant might not be clinically or quantitatively important if a control sample is being evaluated. However, a matrix effect of similar magnitude might be of concern if the processed sample were to be used as a calibrator.¹⁶

- (6) **Groups of Interrelated Processed Samples.** If a group of processed samples is interrelated and yields results that demonstrate a persistent bias, even if the results are within the prediction interval, it might be beneficial to continue the analysis. If processed samples are somehow related (for example, within one round of EQA/PT; manufactured from the same formulation, but different batches), then compare the deviations from the line of best fit that has been calculated and drawn from the results of the processed samples with the fresh patient specimen results, either on an aggregate basis or individually. Increasing the number of processed samples, if they are from similar sources, might be of benefit in the evaluation.

6.4 Possible Variations

The following variations to the proposed matrix effect protocols may improve their utility:

- (1) Analyze more than 20 fresh patient specimens if the resulting data and plot do not provide adequate information for evaluation.

NOTE: Analysis of more than 20 fresh patient specimens will provide greater power for identifying the presence of matrix effects. However, the benefits of more samples only increase by the square root of n as the number gets larger.

- (2) If the scatter around the regression line appears neither proportional nor constant to analyte concentration (i.e., neither variance divided by concentration nor variance itself is constant across analyte concentration), segment the data into several groups of smaller concentration intervals, and perform linear regression analysis within each interval. For example, one would analyze concentration ranges in which the scatter of results from fresh patient specimens about the regression line appears to be approximately constant. A minimum of ten fresh patient specimens that bracket the concentrations of the processed samples should be used within each segment.

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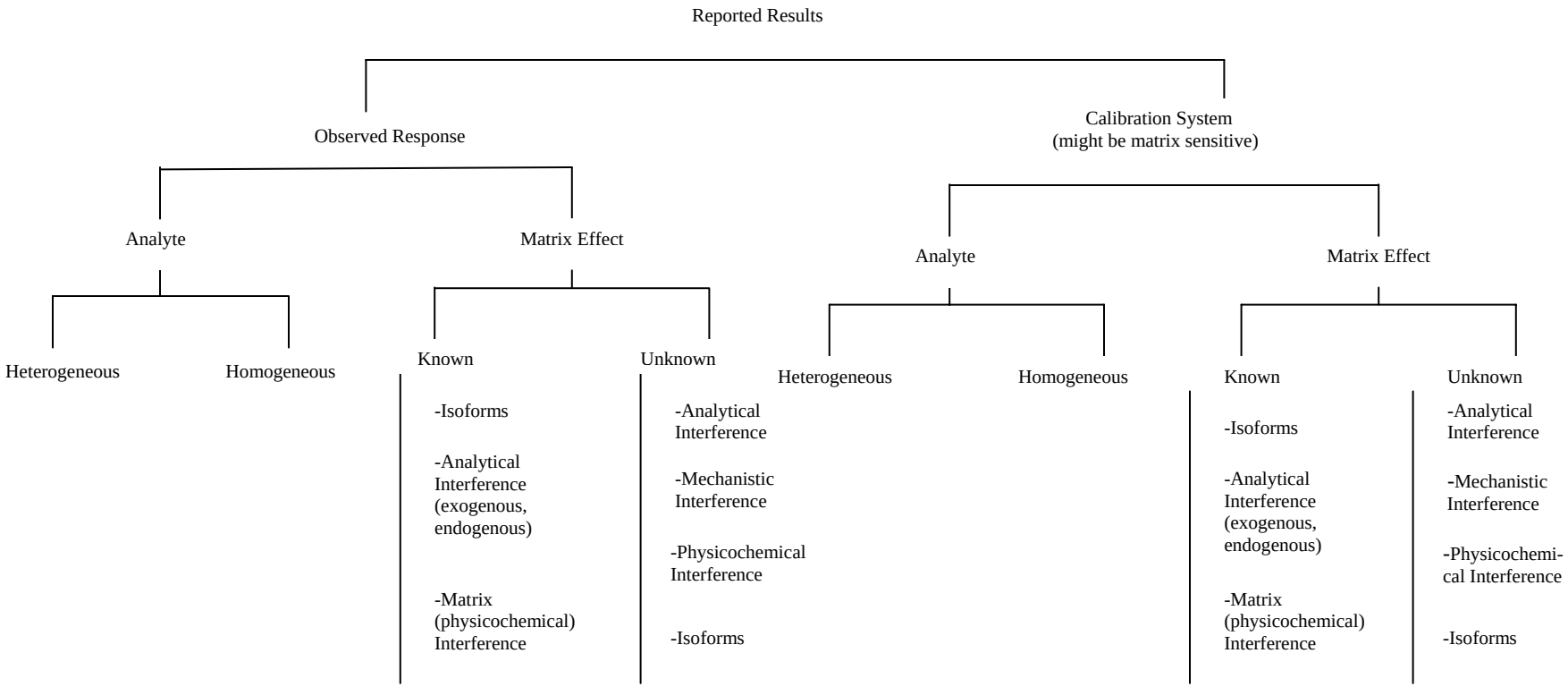
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Appendix A. A Hierarchical Diagram of Factors Affecting Reported Results



Appendix B. Data Input Form

	Comparative			Method Results		
	#1	#2	#3	#1	#2	#3
Processed Sample #1						
Fresh Patient #1						
Fresh Patient #2						
Fresh Patient #3						
Fresh Patient #4						
Fresh Patient #5						
Processed Sample #2						
Fresh Patient #6						
Fresh Patient #7						
Fresh Patient #8						
Fresh Patient #9						
Fresh Patient #10						
Processed Sample #3						
Fresh Patient #11						
Fresh Patient #12						
Fresh Patient #13						
Fresh Patient #14						
Fresh Patient #15						
Processed Sample #4						
Fresh Patient #16						
Fresh Patient #17						
Fresh Patient #18						
Fresh Patient #19						
Fresh Patient #20						
Processed Sample #5						

Analyte:

Units:

Evaluated method:

Comparative method:

Appendix C. Examples of Completed Analyses

Example 1: Cholesterol; Use of Linear Regression Analysis (see Section 6.3(2))

	Method Results	
	Comparative*	Evaluated*
Processed Sample #1	229.6	252
Fresh Patient #1	246.6	245
Fresh Patient #2	194.9	195
Fresh Patient #3	267.9	268
Fresh Patient #4	279.3	281
Fresh Patient #5	182.3	190
Processed Sample #2	161.7	188
Fresh Patient #6	249.2	252
Fresh Patient #7	115.5	116
Fresh Patient #8	181.9	182
Fresh Patient #9	219.1	218
Fresh Patient #10	128.7	136
Processed Sample #3	240.8	265
Fresh Patient #11	148.3	148
Fresh Patient #12	230.2	230
Fresh Patient #13	273.7	265
Fresh Patient #14	159.9	161
Fresh Patient #15	187.8	187
Processed Sample #4	149.2	173
Fresh Patient #16	105.9	107
Fresh Patient #17	176.4	176
Fresh Patient #18	202.3	207
Fresh Patient #19	210.0	211
Fresh Patient #20	204.4	205
Processed Sample #5	179.5	197

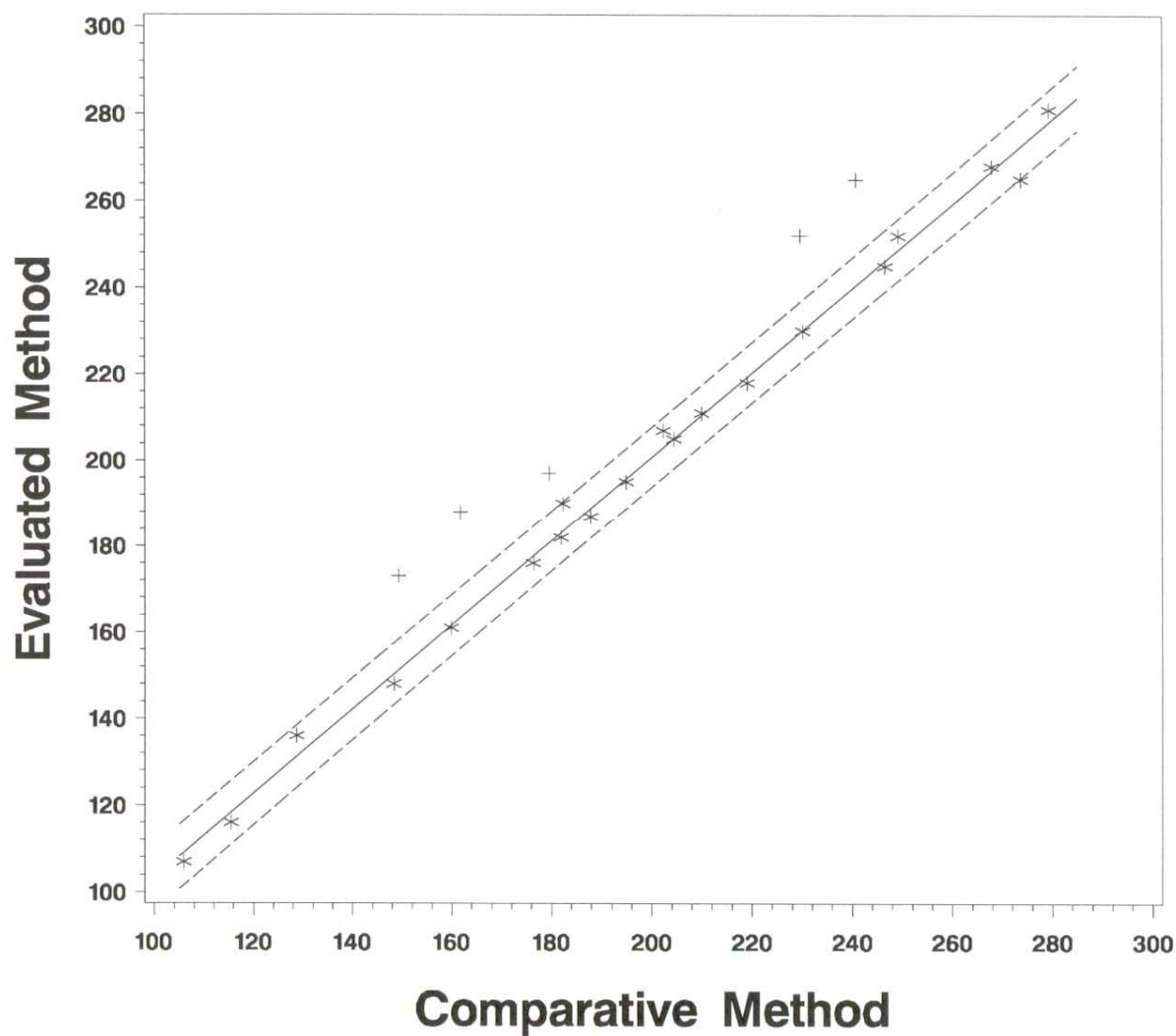
Analyte: Cholesterol

Units: mg/dL

Evaluated method:

Comparative method:

*mean of three replicates

Appendix C. (Continued)**Example 1: Sample Plot for Cholesterol**

Conclusion: Processed samples exhibit matrix effects that are different from the patient specimens.

NOTE:

- * Represents fresh patient specimens
- + Represents processed samples

Appendix C. (Continued)

Example 2: Salicylate: Use of Polynomial Regression Analysis (see Section 6.3(2))

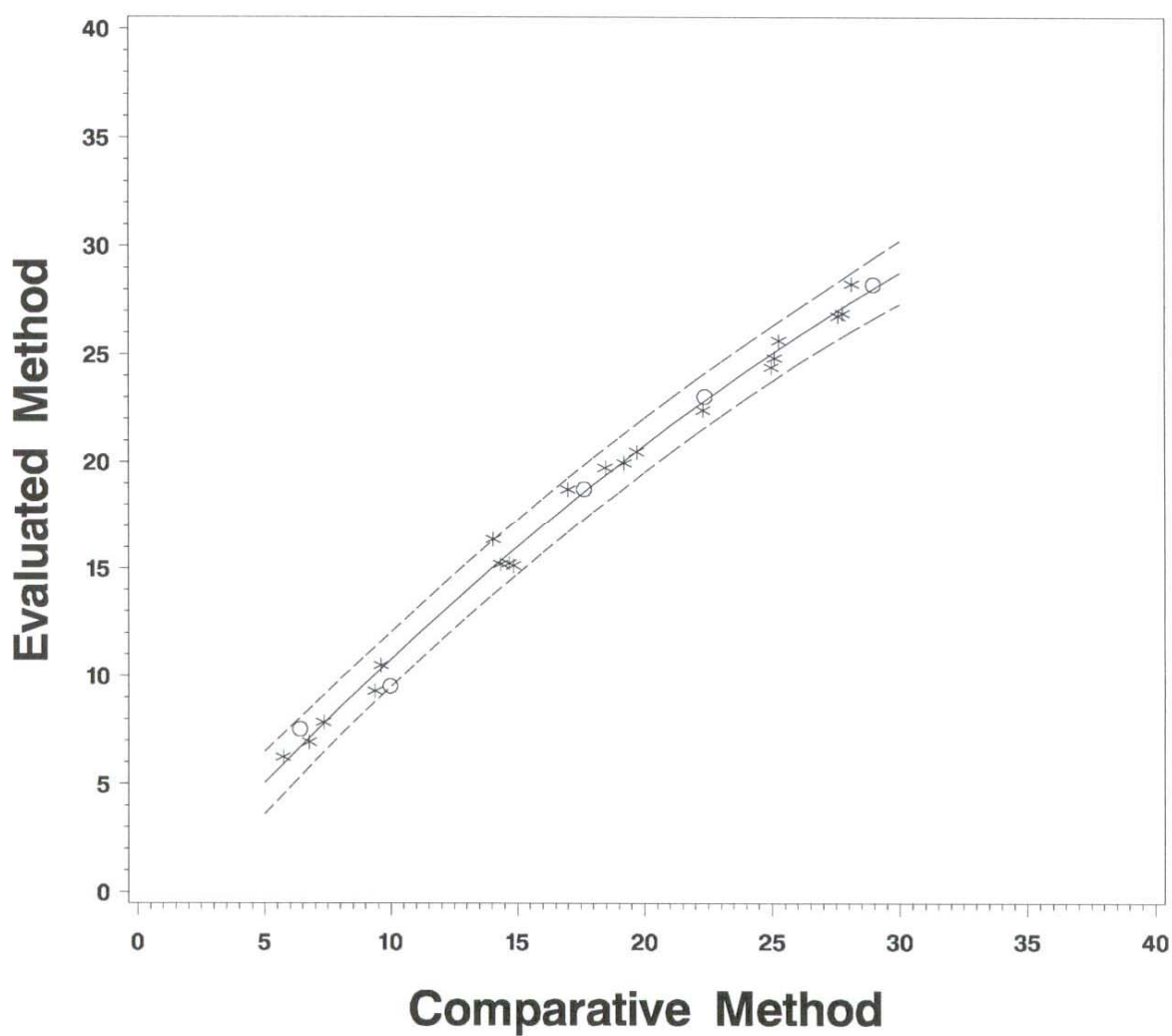
	Method Results					
	Comparative			Evaluated		
	#1	#2	#3	#1	#2	#3
Processed Sample	28.4	29.1	29.4	28.3	28.0	28.4
Fresh Patient #1	27.8	27.7	27.8	27.2	26.9	26.6
Fresh Patient #2	9.6	9.6	8.9	9.6	9.1	9.2
Fresh Patient #3	28.0	28.4	28.0	27.2	29.0	28.6
Fresh Patient #4	7.7	6.9	7.4	8.1	7.8	7.7
Fresh Patient #5	27.4	27.8	27.6	27.7	26.4	26.2
Processed Sample #2	23.0	20.9	23.2	23.4	22.8	22.9
Fresh Patient #6	26.0	24.2	25.6	25.4	25.8	25.7
Fresh Patient #7	25.1	25.1	25.1	24.6	25.6	24.3
Fresh Patient #8	9.6	9.4	9.8	10.6	10.7	10.2
Fresh Patient #9	17.0	16.1	17.9	18.7	18.8	18.7
Fresh Patient #10	25.1	24.4	25.4	24.4	24.4	24.4
Processed Sample #3	18.9	16.1	17.9	18.7	18.8	18.7
Fresh Patient #11	22.4	22.4	22.1	22.5	22.4	22.3
Fresh Patient #12	19.7	19.0	20.4	20.8	20.5	20.1
Fresh Patient #13	20.8	17.7	19.1	20.2	20.0	19.6
Fresh Patient #14	15.3	13.3	14.4	15.2	15.1	15.3
Fresh Patient #15	18.5	18.4	18.5	20.4	19.9	18.9
Processed Sample #4	9.3	11.5	9.1	9.7	9.4	9.5
Fresh Patient #16	14.5	15.0	14.5	14.8	15.8	15.1
Fresh Patient #17	6.9	6.6	6.8	7.1	6.9	6.8
Fresh Patient #18	12.4	12.1	20.0	12.5	17.0	15.9
Fresh Patient #19	5.8	5.8	5.6	6.3	6.5	5.9
Fresh Patient #20	13.1	15.8	13.2	16.2	17.0	15.9
Processed Sample #5	6.5	6.8	5.9	7.8	7.6	7.2

Analyte: Salicylates

Units: mg/dL

Evaluated method:

Comparative method:

Appendix C. (Continued)**Example 2: Sample Plot for Salicylate**

Conclusion: Processed samples do not exhibit matrix effects.

NOTE:

- * Represents fresh patient specimens
- + Represents processed samples

CLSI consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact the Executive Offices or visit our website at www.clsi.org.

Summary of Consensus/Delegate Comments and Committee Responses

EP14-A2: *Evaluation of Matrix Effects; Approved Guideline—Second Edition*

General

1. The acknowledgement states members and advisors of the original subcommittee; however, the advisors are left off.
- **The Acknowledgement has been revised to indicate the prime contributors to the original document.**

Section 4, Definitions

2. I recommend using the ISO definition of commutability to be globally consistent.
- **The definition has been updated to be consistent with EN 12287:1999, and referenced in ISO 15197 and ISO 15193.**
3. For the definition of specificity, I suggest deleting note A) regarding QC, as “false alarm” implication is not applicable to this document.
- **The committee agrees. The statement with reference to Quality Control is not applicable and has been deleted.**

Section 5, Principle of Evaluation

4. First sentence: “actual activity or concentration is dependent...” Change “is” to “may be.”
- **This sentence has been revised to read: “The evaluation of a matrix effect is based on the principle that the relationship between an observed response and the actual activity or concentration is often dependent upon the environmental conditions...”**
5. Paragraph 3. The parenthetical statement reads, “the regression techniques used in these protocols use the assumption that there is no error in the comparative method...” This assumption is predominantly incorrect with most comparative methods. Instructions should be given as to how to reduce the error in the comparative method to acceptable levels.
- **The statement is correct as written for the statistical models used here. The statement does not refer to procedural error of either method, but to the statistics used. A phrase has been added to make it more evident that every method has error and that the protocols contained in this document reduce errors associated with imprecision through the use of triplicate measurements. However, imprecision cannot be completely eliminated. Additionally, only the errors due to differences in specificity are isolated for evaluation using these protocols. Since each measurement procedure has its own sources of error, we caution that the protocols in EP14 might not be effective in determining which of the two methods is superior.**

Section 6.1, Materials

6. Under the second bullet, NRSCL definitions for definitive method and reference method have been superseded by JCTLM and ISO nomenclature “reference measurement procedure.” For global acceptance the current metrological nomenclature and definitions should be used throughout the document.
- **The committee agrees. As CLSI/NCCLS documents are approved and reapproved, current acceptable or agreed to nomenclature should be used, when possible, in the spirit of harmonization. The unofficial modifiers (“primary” and “secondary”) have been added to “reference measurement procedure” to help distinguish the relative metrological order of the examples. We are also aware that the examples of isotope dilution-mass spectrometry (ID/MS) and Abell-Kendall (A-K) are not fully consistent with current designation (they are both considered “reference measurement procedures”), but most chemists agree that ID/MS is probably superior, while the A-K method is excellent, but biased with regard to “trueness.”**

7. Under the third bullet, the “e.g.” list should include “reference materials” because this document is directly applicable to those materials, and those materials and their commutability are quite important for correct application of traceability.
- **This material has been added to the list of materials for evaluation. The committee agrees that the procedures can be used to demonstrate commutability, albeit the rigor of the test (more fresh patient specimens, more reagent and calibrator lots, more runs) would need to be increased significantly.**

Section 6.2, Procedure

8. (3): I suggest adding “same” before “20 fresh” to be consistent with the next phrase for processed samples.
- **The suggested revision has been made.**
9. (4): Any effects due to freezing the sample must be understood prior to the freezing and subsequent retesting.
- **The last sentence in the paragraph contains the appropriate cautionary statement.**

Section 6.3, Data Analysis

10. (1) & (2): EP9 uses an N of 40 for regression statistics. With an N of only 20, the prediction intervals will be larger and the ability to detect effects will be reduced.
- **There is no prohibition to using more specimens or replicates. The number of specimens is a judgment of the experimenter that should be based on the problem to be addressed and criteria for acceptance. Although more specimens would increase the user’s confidence in the results, the principle of diminishing returns needs to be considered.**
11. (2): There is inconsistency and confusing language in the last paragraph of Linear Regression Analysis where it states, “using the means of the evaluated results” vs. the first paragraph in Polynomial Regression Analysis which states to use “the values of the evaluated method results as the y values and the means of the comparative method results as the x values.” This language is confusing to the reader to know exactly what is correct. The use of the “mean” continues on page 8 in paragraph (4). It would be better to state (as it already is) that each data point for analysis is the mean of the triplicate measurements and then use a term such as “data point” to be clear what is being referred to and plotted and regressed. This terminology gets very confusing in paragraph (4).
- **The first paragraph under “Polynomial Regression Analysis” has been revised to read: “The linearity of the evaluated method results and the comparative method results for the fresh patient specimens should be checked using procedures outlined in CLSI/NCCLS document EP6—*Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*, using the mean values of replicate testing from each method and including calculation of the best fitting polynomial regression model.”**
12. (2): In Section 6.3, it states that linear regression analysis should be performed on the means of the evaluated method results versus the means of the comparative method results. However, under Polynomial Regression Analysis it refers to “the values of the evaluated method results as the y values and the means of the comparative method results as the x value.” Interpretation of this statement is that the individual results for the evaluated method (three per patient sample) are each being paired with the mean of the three replicates for the comparative method. Is that what is intended? It is the way to proceed if one were going to test for model lack of fit, because the replicates allow estimation of a pure error SD that can be used for testing lack of fit, but it is unclear if that is what is being proposed here.
- **The sentence has been revised to read: “As an example, if the best fitted polynomial is a second-order polynomial with the mean of the values of the evaluated method results as the y values and the means of the comparative method results as the x values (see Appendix C, Example 2), then:”**
13. On page 8, replace “reference method” with “reference measurement procedure.”
- **The suggested revision has been made.**
14. (3): This paragraph requires a value judgment on the part of the analyst but no guidelines are given for making the judgment if the “regression line seems to increase in proportion to the measurand concentration.” I suggest providing criteria or a statistical test to make this value judgment. Perhaps a log transformation can be made and criteria to determine if the scatter of that fit is an improvement over a linear plot. Can other transformations also be made with acceptable data analysis results? This paragraph needs reworking or possible deletion if the statistical considerations get excessively complex.

- **This is a common concern with descriptive statistics, that is, which model is appropriate for analysis of data. It is common to review data (usually in graphical format) and use subjective reasoning to decide, based, of course, on sound scientific judgment and experience. The suggestion is not accepted. More importantly, the use of these protocols by manufacturers to determine the presence (or absence) of matrix effects has been used effectively.**
15. (3): Regarding the statement, “If the scatter of patient specimen results around the regression line seems to increase in proportion to the measurand concentration, rather than being constant across the concentration range (i.e., variance divided by concentration is constant...).”
- It seems that scatter around the regression that increases proportional to concentration implies that the standard deviation (square root of the variance) divided by concentration is constant, not the variance divided by concentration. A similar statement is made at the bottom of page 9.
- **The committee agrees, and “variance” has been changed to “standard deviation.” To determine whether or not a transformation is necessary or preferred, the user must judge the uniformity of the standard error or regression ($S_{y,x}$) across the range of concentrations.**
16. (4): The first sentence, “for the mean of the fresh patient specimens” is confusing. I suggest replacing with “data point” defined as the mean of triplicate measurements (previously suggested in an earlier comment). Also, delete the word “mean” in parentheses following “ $\bar{X}_i$ ” and “ $\bar{Y}_i$ ” definitions for the terms in the equations because it is confusing to a reader.
- **The text has been edited for clarity. The descriptions of symbols for the equation in (4) are judged to be clear as written.**
17. (4): The wording is unclear how to generate the dashed line in the Appendix examples. I suggest adding a sentence to the first paragraph: “The prediction interval for the y values is calculated for a series of x values and plotted on the graph along with the regression line.”
- **The suggested statement has been added.**
18. (4): In the definition of the term “ $\bar{X}$ double bar,” change “reference method” to “comparative method” to reflect the more generic term for the x-axis method, since a reference method may not always be available.
- **The suggested correction has been made.**
19. Formula (2) on page 8 is only appropriate for linear regression (either on the means or the log-transformed means), but is not appropriate for polynomial regression. The formula for a second-order polynomial regression line is much more complicated than formula (2). It does not appear that this formula was used to calculate the confidence interval in example 2. The formula for $S_{y,x}$ also is not correct for polynomial regression. The denominator should be $n-3$ for a second-order polynomial.
- **The comment is correct, but the difference between this formula and the correct one for a second order polynomial is not important for the purposes of this document; it would add needless complexity. The number of degrees of freedom was changed to 2 or 3, depending on the order of the regression (linear or quadratic). The calculations in Example 2 use this formula.**
20. Recommending log-transforming the means of the evaluated method and comparative method if scatter appears proportional to concentration seems to be a less than optimal approach. While the transformation will make the scatter more constant, if the linear relationship does not have an intercept equal to zero, the transformation will also induce curvature into the relationship. In most cases, we would expect an intercept near zero so maybe it isn’t an issue, but there is an alternative approach that seems just as easy and also makes the scatter constant, but doesn’t induce curvature if the intercept differs from zero. It involves fitting y/x versus $1/x$ by linear regression, calculating the predicted y/x and confidence interval (using equation 2 with $1/x$ in place of x) and then plotting $(\text{predicted } y/x) \cdot x$ and $(\text{Confidence interval curves for } y/x) \cdot x$ versus x . This is effectively performing a weighted least squares regression using weights equal to $1/x^2$.
- **This is a novel technique, and it might be useful in some situations, so it should be investigated in a future revision of EP14 with examples to illustrate its applicability. As the commenter states, it should not have much benefit in most cases, and so does not compel major revision of this document.**
21. (5): The last sentence and the text in the first Note should be deleted. The concept of independent evaluation criteria is out of place in this document. The identification of a matrix bias by statistical testing is the purpose of this guideline. Evaluation of the magnitude of the matrix bias is out of scope. Furthermore, matrix bias is a confounding factor in interpreting a PT result in that the matrix bias can make the observed bias for a PT material an incorrect representation of the actual bias for patient specimens for that method.

- The commenter misses the point of this sentence for two special cases (and perhaps others). First, processed material can exhibit a statistically verified matrix effect, but still be acceptable for evaluating comparability of two methods. This occurs frequently when methods are exquisitely precise, very specific when patient specimens are measured and the total allowable error for clinical use of the measured substance is relatively large (compared to the precision/specificity of both methods). In such cases, the candidate processed material might still serve as adequate controls for evaluation or comparison.

The other example is when specific criteria are established for accuracy, such as for total cholesterol, and processed materials demonstrate consistent biases (matrix effects) that are within the prediction interval of patient specimens. For example (using percent for simplicity of discussion), a set of processed materials used for evaluation of a commercially available cholesterol method produces a small, consistent +1.5% bias when the routine method results are compared to the Abell-Kendall reference measurements. The prediction interval from fresh patient samples is $\pm 3.8\%$, therefore, according to these protocols, the materials are deemed as acceptable surrogates for fresh specimens. However, with a systematic matrix effect, the routine method is put at a significant disadvantage when it is evaluated with these materials when an independent acceptability criteria of $\pm 5\%$ is used, because the allowable error for the method has been reduced to the remaining +3.5% (and expanded to -6.5%). Therefore, these procedures must be used with keeping the intent of the programs in mind.

The note is a useful reminder that PT (EQA) samples, when run in singlicate (as is required under CLIA Regulation), do not conform to the requirements of these procedures. Evaluating the adequacy of a method using external proficiency surveys when matrix effects were present was the impetus for development of this guideline. The Note, therefore, remains relevant.

22. (6): This paragraph should be deleted. The statements are vague, and there is no published literature to support the claim that the same or similar matrix bias is observed in processed samples related by an admixture relationship of a low and high concentration pool. Several abstracts and Edutracks presented at AACC 2004 show that processed samples from an interrelated series have different matrix biases for a given analyte. These data will be published in Archives of Pathology and Laboratory Medicine in the near future.
- The paragraph is clear as written. These are suggestions for certain conditions that will enable the user to make an evaluation. Whether such an interrelationship exists can be judged from the recommended protocol. In addition, the evidence that such relationships can exist is empirical, and has been observed by the chair of the committee in support of methods in which national external quality assessment survey materials were used. The data were shared with the appropriate organizations that conduct the respective surveys. The information was not deemed of sufficient importance for publication, because the outcomes were clearly predictable: the matrix effects varied as a function of the admixture of samples used to prepare sets of evaluation material. None the less, the statement is helpful in light of the cautions presented in paragraph (5).

References

23. One recent review that should be included is: Miller WG. Specimen materials, target values and commutability for external quality assessment (proficiency testing) schemes. *Clin Chim Acta*. 2003;327:25-37.
- This reference has been added as part of a new discussion on commutability that appears in the Foreword. However, we are concerned that the author of this article references this guideline and procedures (i.e., “A consensus procedure to document a material’s commutability with authentic clinical specimens is available in NCCLS document EP14-A”) but overstates the intent of the document indicated in the scope. The suggestion to consider this document as a means to that end is good, and worthy in our estimation, and the reference has been included with the further recommendation indicated in the response to comment 7, above, that if these procedures were to be used to assess the commutability of material, the robustness of the procedure would need to be increased, because of the implications to traceability and accuracy of patient results. It is gratifying to see that the work of the committee is recognized as useful.

NOTES

The Quality System Approach

CLSI subscribes to a quality 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 approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*. The quality system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any healthcare service’s path of workflow (i.e., 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 quality system essentials (QSEs) are:

Documents & Records
Organization
Personnel

Equipment
Purchasing & Inventory
Process Control

Information Management
Occurrence Management
Assessment

Process Improvement
Service & Satisfaction
Facilities & Safety

EP14-A2 addresses the quality system essentials (QSEs) indicated by an “X.” For a description of the other CLSI/NCCLS documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
					X EP5 EP6 EP7 EP9 I/LA15						M29

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*.

Related CLSI/NCCLS Publications*

EP5-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 for 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.
EP6-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.
EP7-A	Interference Testing in Clinical Chemistry; Approved Guideline (2002). This guideline provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interfering substances on clinical chemistry test results.
EP9-A2	Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (2002). This document addresses procedures for determining the bias between two clinical methods or devices, and for the design of a method comparison experiment using split patient samples and data analysis.
I/LA15-A	Apolipoprotein Immunoassays: Development and Recommended Performance Characteristics; Approved Guideline (1997). This guideline describes the characterization and preparation of immunogens, antibodies, samples, and methods, and provides guidance for immunochemical testing of apolipoproteins.
M29-A2	Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Second Edition (2001). This document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.
NRSCL13-A	The Reference System for the Clinical Laboratory: Criteria for Development and Credentialing of Methods and Materials for Harmonization of Results; Approved Guideline (2000). This document contains procedures for developing and evaluating definitive methods, reference methods, designated comparison methods, and reference materials to provide a harmonized clinical measurement system.

* Proposed- and tentative-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most recent editions.

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