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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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Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: 610.688.0100
F: 610.688.0700
www.clsi.org
standard@clsi.org

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Evaluation of Matrix Effects; Approved Guideline—Second Edition

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Fred D. Lasky, PhD
Martin Harris Kroll, MD
Daniel W. Tholen, MS

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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Committee Membership

Area Committee on Evaluation Protocols

Jan S. Krouwer, PhD
Chairholder
Krouwer Consulting
Sherborn, Massachusetts

Luann Ochs, MS
Vice-Chairholder
Roche Diagnostics Corporation
Indianapolis, Indiana

Anders Kallner, MD, PhD
Karolinska Hospital
Stockholm, Sweden

Martin Harris Kroll, MD
Dallas VA Medical Center
Dallas, Texas

Jacob (Jack) B. Levine, MBA
Bayer Corporation
Tarrytown, New York

Kristian Linnet, MD, PhD
Psychiatric University Hospital
Risskov, Denmark

Kristen L. Meier, PhD
FDA Center for Devices and
Radiological Health
Rockville, Maryland

Max Robinowitz, MD
FDA Center for Devices and
Radiological Health
Rockville, Maryland

Daniel W. Tholen, MS
Dan Tholen Statistical Consulting
Traverse City, Michigan

Advisors

David A. Armbruster, PhD,
DABCC, FACB
Abbott Laboratories
Abbott Park, Illinois

R. Neill Carey, PhD
Peninsula Regional Medical Center
Salisbury, Maryland

Carl C. Garber, PhD, FACB
Quest Diagnostics, Incorporated
Teterboro, New Jersey

Patricia E. Garrett, PhD
Boston Biomedica, Inc.
West Bridgewater, Massachusetts

Donald M. Powers, PhD
Powers Consulting Services
Pittsford, New York

Gian Alfredo Scassellati, PhD
Ente Nazional Italiano Di
Unificatione
Turin, Italy

Jack Zakowski, PhD
Beckman Coulter, Inc.
Brea, California

Staff

Clinical and Laboratory Standards
Institute
Wayne, Pennsylvania

Lois M. Schmidt, DA
Staff Liaison

Donna M. Wilhelm
Editor

Melissa A. Lewis
Assistant Editor

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Fred D. Lasky, PhD, Chairholder
Donald Joe Boone, PhD
John H. Eckfeldt, M.D., PhD
Carolyn S. Feldkamp, PhD
David J. Hassemer, MS
Sandy Krishnamurthy, MS
Thomas A. Long, MPH
W. Greg Miller, PhD
Herbert K. Naito, PhD, MBA
Alan Posner, PhD

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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