CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

EP34

Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking

It is often medically necessary to provide results for specimens with concentrations above the analytical measuring interval of an *in vitro* diagnostic measurement procedure. This guideline helps manufacturers and laboratory scientists with establishing, validating, or verifying a dilution scheme that will provide an extended measuring interval for such specimens.

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

Clinical and Laboratory Standards Institute

Setting the standard for quality in medical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advances in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential and may be submitted by anyone, at any time, on any document. All comments are managed according to the consensus process by a committee of experts.

Appeal Process

When it is believed that an objection has not been adequately considered and responded to, the process for appeal, documented in the CLSI *Standards Development Policies and Processes,* is followed.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

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

Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org

EP34, 1st ed.
August 2018

Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking

Jeffrey R. Budd, PhD
Yu Chen, MD, PhD, DABCC, FACB
Iwona Fijalkowska, PhD
Mahdi Garelnabi, MSc, MLT, PhD
Abdel-Baset Halim, PharmD, PhD, DABCC, FACB
Jason A. Kellogg, PhD
George G. Klee, MD, PhD
Teemu Korpimäki, PhD
Heather Kuiper, PhD
Mark J. Magera, MA
David Sogin, PhD
Susan Lanning Taylor, MS, MBA
John G. Toffaletti, PhD
Anca Roxana Varlan, PhD
Linda Xu, PhD

Abstract

Clinical and Laboratory Standards Institute guideline EP34—*Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking* provides recommendations for establishing a dilution scheme to be used for patient specimens that contain measurand concentrations in the extended measuring interval above a measurement procedure's upper limit of quantitation. Guidance is provided on determining, validating, and verifying the appropriate diluent and dilution ratio to be used for such specimens. This guideline also covers creating spiked samples for use during dilution recovery studies and using spiking to determine the suitability of a sample matrix for dilution recovery studies. The intended users of this guideline are manufacturers of *in vitro* diagnostic tests and medical laboratory scientists, directors, and pathologists.

Clinical and Laboratory Standards Institute (CLSI). *Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking*. 1st ed. CLSI guideline EP34 (ISBN 978-1-68440-014-0 [Print]; ISBN 978-1-68440-015-7 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2018.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Suggested Citation

CLSI. *Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking*. 1st ed. CLSI guideline EP34. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

ISBN 978-1-68440-014-0 (Print)

ISBN 978-1-68440-015-7 (Electronic)

ISSN 1558-6502 (Print)

ISSN 2162-2914 (Electronic)

Volume 38, Number 16

Committee Membership

Consensus Council

Dennis J. Ernst, MT(ASCP), NCPT(NCCT)
Chairholder
Center for Phlebotomy Education
USA

Mary Lou Gantzer, PhD, FACB
Vice-Chairholder
USA

J. Rex Astles, PhD, FACB, DABCC
Centers for Disease Control and Prevention
USA

Lucia M. Berte, MA, MT(ASCP)SBB, DLM, CQA(ASQ)CMQ/OE
Laboratories Made Better!
USA

Karen W. Dyer, MT(ASCP), DLM
Centers for Medicare & Medicaid Services
USA

Thomas R. Fritsche, MD, PhD, FCAP, FIDSA
Marshfield Clinic
USA

Loralie J. Langman, PhD, DABCC, FACB, F-ABFT
Mayo Clinic
USA

Ross J. Molinaro, PhD, MLS(ASCP)CM, DABCC, FACB
Siemens Healthcare Diagnostics, Inc.
USA

James R. Petisce, PhD
BD Diagnostic Systems
USA

Andrew Quintenz
Bio-Rad Laboratories, Inc.
USA

Robert Rej, PhD
New York State Department of Health – Wadsworth Center
USA

Zivana Tezak, PhD
FDA Center for Devices and Radiological Health
USA

Document Development Committee on Characterization of Dilution and Spiking Recovery

Jeffrey R. Budd, PhD
Chairholder
Beckman Coulter
USA

Yu Chen, MD, PhD, DABCC, FACB
Horizon Health Network
Canada

Iwona Fijalkowska, PhD
FDA Center for Devices and Radiological Health
USA

Mahdi Garelnabi, MSc, MLT, PhD
University of Massachusetts Lowell
USA

Jason A. Kellogg, PhD
Siemens Healthcare Diagnostics, Inc.
USA

George G. Klee, MD, PhD
Mayo Clinic
USA

Teemu Korpimäki, PhD
PerkinElmer
Finland

Heather Kuiper, PhD
Centers for Disease Control and Prevention
USA

John G. Toffaletti, PhD
Duke University Medical Center
USA

Staff

Clinical and Laboratory Standards Institute
USA

Luann Ochs, MS
Project Manager

Megan L. Tertel, MA, ELS
Editorial Manager

Catherine E.M. Jenkins
Editor

Kristy L. Leirer, MS
Editor

Laura Martin
Editor

Acknowledgment for the Expert Panel on Evaluation Protocols

CLSI, the Consensus Council, and the Document Development Committee on Characterization of Dilution and Spiking Recovery gratefully acknowledge the Expert Panel on Evaluation Protocols for serving as technical advisors and subject matter experts during the development of this guideline.

Expert Panel on Evaluation Protocols

James H. Nichols, PhD, DABCC, FACB
Chairholder
Vanderbilt University School of Medicine
USA

Nils B. Person, PhD, FACB
Vice-Chairholder
Siemens Healthcare Diagnostics, Inc.
USA

Valeria L. Alcon, PhD
Health Canada
Canada

Jeffrey R. Budd, PhD
Beckman Coulter
USA

Mark D. Kellogg, PhD
Children's Hospital Boston
USA

Marina V. Kondratovich, PhD
FDA Center for Devices and Radiological Health
USA

Paula Ladwig, MS, MT(ASCP)
Mayo Clinic
USA

Robert J. McEnroe, PhD
Roche Diagnostics, Inc.
USA

Curtis A. Parvin, PhD
Bio-Rad Laboratories, Inc.
USA

Acknowledgments

CLSI, the Consensus Council, and the Document Development Committee on Characterization of Dilution and Spiking Recovery gratefully acknowledge the following volunteers for their important contributions to the development of this guideline:

Abdel-Baset Halim, PharmD, PhD, DABCC, FACB
Celldex Therapeutics
USA

Mark J. Magera, MA
Mayo Clinic
USA

David Sogin, PhD
USA

Susan Lanning Taylor, MS, MBA
Diagnostica Stago
USA

Anca Roxana Varlan, PhD
Epocal, an Alere Company
Canada

Linda Xu, PhD
Theranos
USA

CLSI, the Consensus Council, and the Document Development Committee on Characterization of Dilution and Spiking Recovery gratefully acknowledge the following special reviewers for their valuable input:

Karl De Vore
Bio-Rad Laboratories, Inc.
USA

Robert J. McEnroe, PhD
Roche Diagnostics Corporation
USA

James H. Nichols, PhD, DABCC, FACB
Vanderbilt University Medical Center
USA

Gene Pennello, PhD
FDA Center for Devices and Radiological Health
USA

James F. Pierson-Perry
Siemens Healthcare Diagnostics, Inc.
USA

Megan E. Sawchuk, MT(ASCP)
Centers for Disease Control and Prevention
USA

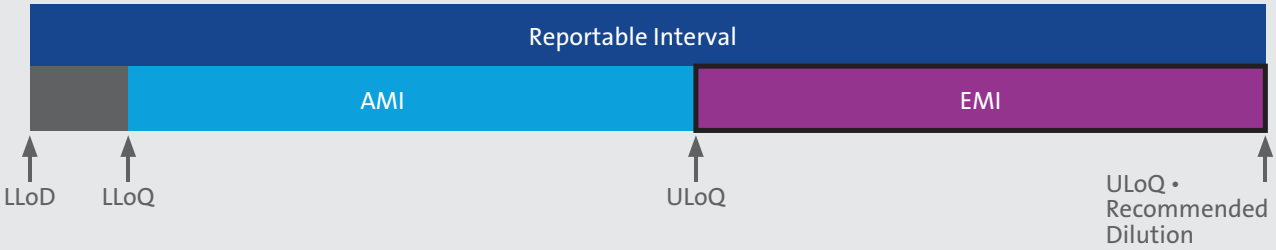
Mitchell G. Scott, PhD
Barnes-Jewish Hospital
USA

Contents

Abstract i
Committee Membership iii
Foreword ix
Chapter 1: Introduction 1
1.1 Scope 2
1.2 Standard Precautions 3
1.3 Terminology 3
Chapter 2: Establishing an Extended Measuring Interval Through Specimen Dilution and Spiking 11
2.1 Dilution Recovery Studies During Measurement Procedure Development 14
2.2 Spiking Studies 31
Chapter 3: Validating an Extended Measuring Interval 45
3.1 General Concerns Regarding Validation of the Extended Measuring Interval 46
3.2 Validating the Dilution Scheme 49
3.3 Dilution Scheme Validation Examples 51
Chapter 4: Verifying the Dilution Process for an Extended Measuring Interval in the Laboratory 57
4.1 Verifying Manual Dilution Recovery Claims 58
4.2 Verifying Automated Dilution Processes 58
Chapter 5: Conclusion 61
Chapter 6: Supplemental Information 63
References 64
Appendix A. Analytical Measuring Interval and Reportable Interval Example 66
Appendix B. Dilution Computational Equations 68
Appendix C. Examples of Completed Dilution Analyses 70
Appendix D. Effects of Dilution on Heterophilic and Nonheterophilic Interferences 73
Appendix E. Hook Effect vs Extended Measuring Interval 75
The Quality Management System Approach 78
Related CLSI Reference Materials 80

Foreword

Measurement procedures provide measurand results within specified concentration intervals. These intervals are described in Figure 1.



Abbreviations: AMI, analytical measuring interval; EMI, extended measuring interval; LLoD, lower limit of detection; LLoQ, lower limit of quantitation; ULoQ, upper limit of quantitation.

Figure 1. Concentration Intervals

The **analytical measuring interval (AMI)** is the interval in which specimen concentrations are measured within the medical and laboratory needs for accuracy with no dilution, concentration, or other pretreatment not part of the standard or routine measurement process. The AMI includes the interval in which linearity, precision, and bias have been deemed acceptable and extends from the LLoQ to the ULoQ. The **extended measuring interval (EMI)** is the interval in which concentrations are measured with appropriate accuracy by diluting the specimen before taking a measurement with the developed measurement process. The upper limit of this interval is defined by the ULoQ multiplied by the dilution factor recommended in the established dilution scheme. The **reportable interval** includes the AMI and EMI but also extends to the LLoD. An example is included in Appendix A.

Guidance on determining the LLoD and LLoQ of the AMI is available in CLSI document EP17.[1] Guidance on determining the linearity interval is available in CLSI document EP06.[2] There is often great clinical need to provide results for specimens with measurand concentration values above the AMI. This guideline aims to assist manufacturers and laboratory scientists with establishing and verifying dilution schemes created to provide an EMI for such specimens.

Manufacturers typically provide recommendations on how to dilute a high-concentration specimen so its resultant concentration value is within the AMI. Thereafter, the measurand concentration value of the specimen before its dilution can be computed. The recommended dilution scheme should include the appropriate diluent and dilution ratio to ensure accurate dilution recovery. Manufacturers are encouraged to follow this guideline in developing measurement procedure– and measurand-specific dilution schemes. When manufacturers do not provide a dilution scheme that meets the laboratory's needs, the laboratory can use the techniques described in this guideline to determine an appropriate dilution scheme.

NOTE:

The AMI is the interval in which specimen concentrations are measured with appropriate accuracy with no dilution, concentration, or other pretreatment not part of the routine measurement process.

NOTE:

The EMI is the interval in which concentrations are measured with appropriate accuracy by diluting the specimen before taking a measurement.

For some measurement procedures, when a neat specimen is presented, the measuring system uses a process of treatment and conditional dilutions designed to expand the AMI without the need for preexamination dilution commonly used to create an EMI (see Appendix A). For such a measurement procedure, its performance within its AMI should be measured like any standard quantitative procedure. The performance of its internal dilution steps can be tested using some of the procedures provided in this guideline, but the specific testing of an EMI is not necessary unless an examination dilution is also provided as an option.

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

KEY WORDS

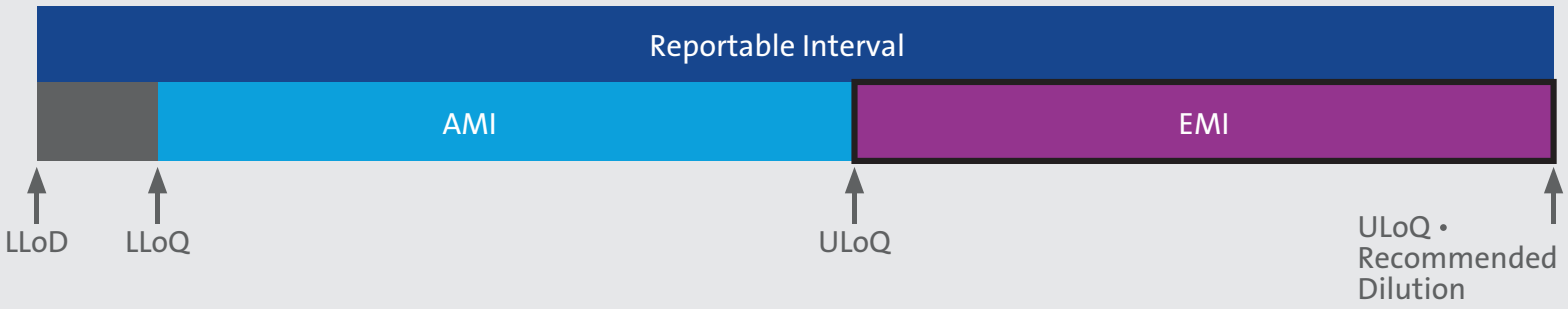
Analytical measuring interval
Diluent
Dilution
Extended measuring interval
Recovery
Spiking
Upper limit of quantitation

Chapter 1

Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's content
- Standard precautions information
- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the guideline
- Abbreviations and acronyms used in the guideline



Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking

1 Introduction

In vitro diagnostic (IVD) device manufacturers often find that to meet clinical needs, a dilution scheme is necessary to measure accurate results beyond the analytical measuring interval (AMI). Establishing and characterizing the dilution scheme using dilution and spiking studies occurs during manufacturers' internal measurement procedure development. This step is followed by validation of the selected dilution scheme and the extended measuring interval (EMI). Finally, the dilution scheme is verified in the laboratory before being used with patient specimens. This wider interval of reportable results provides physicians with important information for screening, diagnosing, monitoring, and treating patients.

1.1 Scope

This guideline provides procedures for establishing, validating, and verifying a dilution scheme to use for obtaining results for patient specimens with measurand concentrations or activity values above a measurement procedure's upper limit of quantitation (ULoQ). This guideline is intended to be used for measurement procedures that have an established AMI within which linearity, precision, and bias have been deemed acceptable. Guidance is provided on determining the appropriate diluent and dilution ratio for these specimens. This guideline also covers creating spiked samples for dilution recovery studies and using spiking studies to determine the suitability of a specimen type for dilution recovery studies. This guideline covers the measurement procedure after it meets design inputs and the resultant AMI has been established. The intended users of this guideline are IVD measuring system manufacturers and medical laboratory scientists, directors, and pathologists.

This guideline does not cover the process of developing a measurement procedure or determining the AMI or interval in which incremental results linearly correspond to increments in a measurand. Thus, it does not cover instituting a set of autonomous dilution steps or reflex examinations as part of the measurement procedure to create accurate results within the AMI. However, it is possible to test the performance of a measuring system's autonomous dilution steps using the protocols described in this guideline.

NOTE:

This guideline covers an already established measurement procedure with an AMI within which acceptable linearity, precision, and bias have been demonstrated.

NOTE:

A measurement procedure may include numerous steps, including dilutions and reflex examinations. However, this guideline does not cover the development of such processes.