



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.

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

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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.

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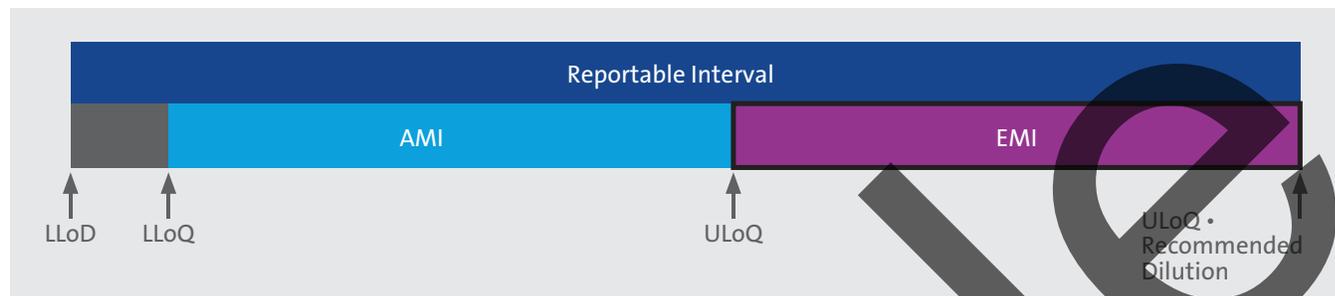
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Foreword

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

Extended measuring interval

Upper limit of quantitation

Diluent

Recovery

Dilution

Spiking

Sample

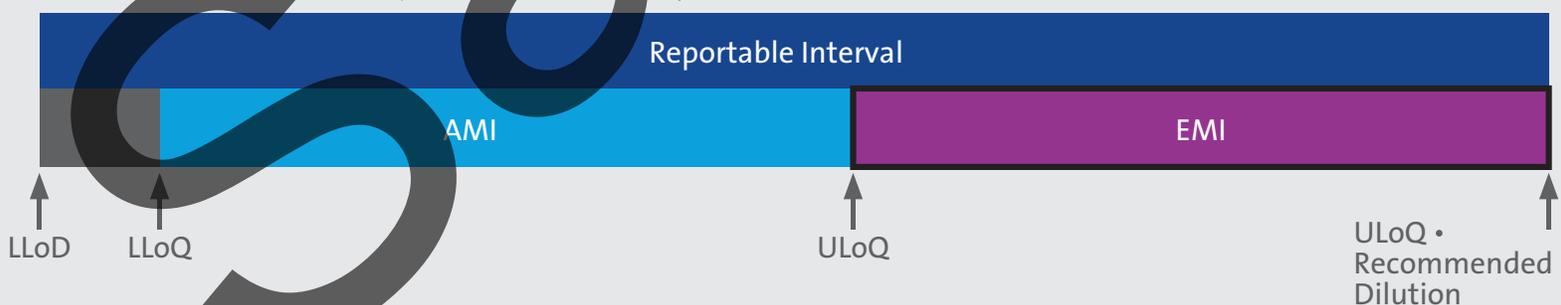
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Chapter 1

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.

Chapter 2

Establishing an Extended Measuring Interval Through Specimen Dilution and Spiking

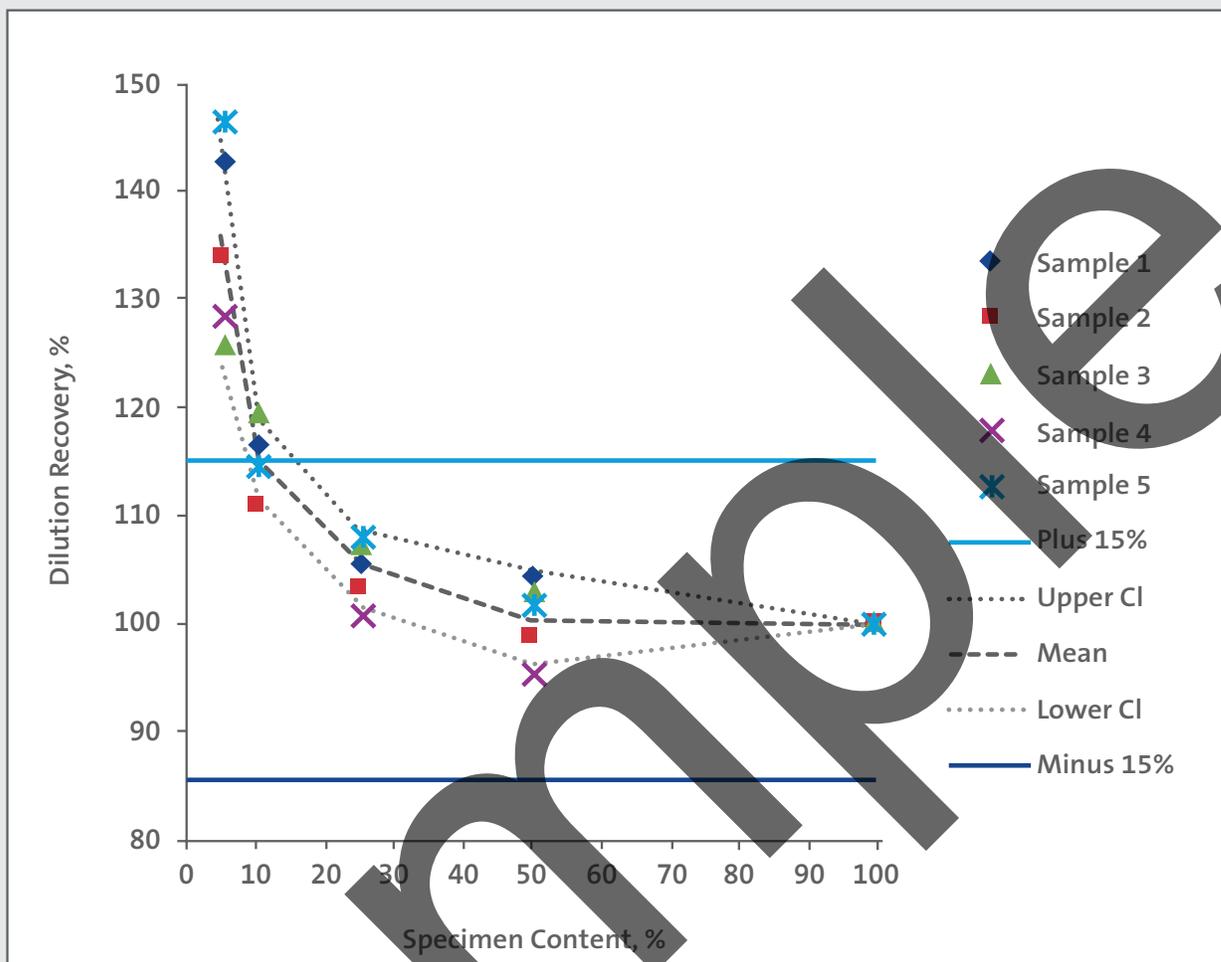
This chapter includes:

- A flow chart indicating the steps for establishing, validating, and verifying an EMI
- Establishing the performance of a dilution scheme for patient specimens during measurement procedure development, including:
 - Determining the appropriate specimen types
 - Determining the appropriate diluent(s)
 - Determining the appropriate dilution ratio
 - Creating and using spiked samples during dilution recovery studies



2 Establishing an Extended Measuring Interval Through Specimen Dilution and Spiking

During measurement procedure development, various performance factors should be characterized, including dilution performance. When an EMI is desired, the appropriate individual components of a dilution scheme (ie, specimen type, diluent, and dilution ratio) should be determined. This guideline provides suggested steps that a measurement procedure developer can take to establish a dilution scheme and to establish measurement procedure performance after implementation of the dilution scheme. Once the developer has confidence in the performance of a dilution scheme, it is tested. When acceptance criteria for dilution recovery are met, the recommended dilution scheme and thus the EMI are validated. Finally, this recommendation (ie, claim) is verified before use in a laboratory setting. The necessary steps for this establishment, validation, and verification are shown in Figure 2, with references to the relevant chapters and subchapters in EP34 included.



Abbreviation: CI, confidence interval.

Figure 7. Multiple Neat Specimen Dilution Plot for Measurand B

When higher dilutions are tested, the dilution series can be plotted using a logarithmic scale on the horizontal axis. This plot provides better discrimination between the high dilutions. In Figure 8, dilutions of 1/2, 1/10, 1/50, and 1/200 are tested and results plotted on a base 10 logarithmic scale. The precision profile within the AMI should be carefully considered per Subchapter 2.1.1.2.1 when high dilution ratios are tested. The data and calculations for producing this plot are in Table C2 in Appendix C.

Related CLSI Reference Materials*

- C50** **Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance. 1st ed., 2007.** This guideline provides a general understanding of mass spectrometry and the principles that dictate its application in the clinical laboratory. It includes guidance, references, and quality assurance markers that will assist with the implementation and correct operation of a mass spectrometry (MS) system for its many applications. Information on maintaining optimum performance, approaches to ensuring accurate and precise mass measurement, verification of methods, quality control of assays within and between instruments, instrument troubleshooting, sample preparation, interpretation of results, and limitations of the technology is included.
- EP05** **Evaluation of Precision of Quantitative Measurement Procedures. 3rd ed., 2014.** This document provides guidance for evaluating the precision performance of quantitative measurement procedures. It is intended for manufacturers of quantitative measurement procedures and for laboratories that develop or modify such procedures.
- EP06** **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. 1st ed., 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.
- EP07** **Interference Testing in Clinical Chemistry. 3rd ed., 2018.** This guideline provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interferences on clinical chemistry test results.
- EP14** **Evaluation of Commutability of Processed Samples. 3rd ed., 2014.** This document provides guidance for evaluating the commutability of processed samples by determining if they behave differently than unprocessed patient samples when two quantitative measurement procedures are compared.
- EP17** **Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. 2nd ed., 2012.** This document provides guidance for evaluation and documentation of the detection capability of clinical laboratory measurement procedures (ie, limits of blank, detection, and quantitation), for verification of manufacturers' detection capability claims, and for the proper use and interpretation of different detection capability estimates.
- M29** **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

Sample



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