CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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EP39

A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of *In Vitro* Medical Laboratory Tests

This guideline establishes a definition of a surrogate sample, provides recommendations for determining when to use surrogate samples, and describes a process for selecting the most appropriate surrogate sample.

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

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A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of *In Vitro* Medical Laboratory Tests

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Abstract

Clinical and Laboratory Standards Institute guideline EP39—*A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of* In Vitro *Medical Laboratory Tests* establishes a standard definition of a surrogate sample. It presents a hierarchical approach for determining when to use surrogate samples and selecting an appropriate one. It also describes elements of a surrogate sample plan and includes technical preparation guidance for the characteristic to be measured or detected and for artificial matrix compositions. This guideline provides examples for specific performance study types.

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Foreword

Terms such as “contrived,” “altered,” “processed,” “diluted,” “supplemented,” and “simulated” have been used interchangeably to describe substitutions for patient samples. This guideline establishes a uniform term, “surrogate sample,” and definition to describe material(s) that is used as a substitute for body fluid or tissue from a single human individual.

When appropriately characterized patient samples are unavailable, surrogate samples serve an important role in the development, validation, and verification of laboratory tests. Surrogate samples may be needed for many reasons, including limited sample volume or inadequate numbers of patient samples with concentrations at medical decision levels or at the extremes of the analytical measuring interval. A lack of available patient samples may be due to low disease prevalence, invasive sampling methods, or other reasons.

This guideline establishes an approach for selecting, preparing, and using surrogate samples. It describes the principles for creating a surrogate sample plan and presents a hierarchy, by performance study type, for selecting an appropriate surrogate sample. The hierarchical approach is demonstrated through product- and performance-specific examples.

KEY WORDS

Artificial analyte
Artificial matrix
Hierarchy
Pooled
Sample plan
Simulated
Supplemented
Surrogate sample

Chapter 1

Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's content
- Standard precautions information
- Terminology information, including:
  - Terms and definitions used in the guideline
  - Abbreviations and acronyms used in the guideline

A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of *In Vitro* Medical Laboratory Tests

1 Introduction

1.1 Scope

This guideline establishes a definition of "surrogate sample" and an approach for selecting, preparing, and using these samples. It discusses surrogate sample:

- Composition
- Technical preparation
- Selection criteria
- Documentation and planning
- Use in specific performance study types

The intended users of this guideline are *in vitro* diagnostic (IVD) device developers, laboratorians, and regulators. This guideline does not describe performance study design, which is covered in other standards and guidelines (see CLSI document EP19[1]).

1.2 Background

Development, validation, and verification of laboratory tests depends on the availability of patient samples for testing. When appropriate patient samples are unavailable to validate test performance, using surrogate samples enables more efficient use of biological materials, improves testing efficiency, and facilitates the development of tests for new biomarkers. Patient samples for test development and other uses may be unavailable for several reasons.

Reasons that patient samples cannot be used include:

- Logistical constraints
- Insufficient sample volumes
- Inadequate numbers of samples, such as those with concentrations at medical decision levels (MDLs) or at the extremes of the analytical measuring interval (AMI)
- Technical constraints
- Unsatisfactory samples (ie, that lack the necessary characteristics for a performance study)
- Instability of samples
- Unavailability of blank or negative samples

3 Samples

3.1 Patient Sample

An analyte sampled for testing originates from the human body, where two components are in balance: the analyte and the surrounding natural matrix. The matrix of the patient sample is all sample components except the analyte. For example, when blood sodium concentrations are measured, sodium is the analyte, while everything else in the blood sample is the matrix. Physical separation methods (eg, centrifugation), use of standard chemical additives in collection devices (eg, EDTA- or heparin-coated tubes), and extraction techniques (eg, those specified in IVD device package inserts) that are necessary to test for the measurand do not alter the designation as a patient sample.

Good medical laboratory practice relies on test methods that were established using patient samples derived from sources that are as close to the intended specimen as possible. For example, in practice, blood and urine specimens are readily obtained from healthy individuals without significant patient risk. When testing an analyte in a patient sample, the developer should be familiar with the most common patient specimens and the optimal maximum time difference between collection and testing. Although the most common patient specimen types depend on the analyte in question and the patient's clinical condition, they generally include (but are not limited to) whole blood, plasma, serum, urine, tissue, nasal swab, and CSF. For many measurands, abundant prior research and literature is available to optimize testing for its intended purposes. The literature supports using particular types of patient specimens, measurand-specific reference intervals, and time intervals for specimen collection. Therefore, when available, patient specimens are the preferred sample type for human clinical studies.

3.2 Surrogate Sample

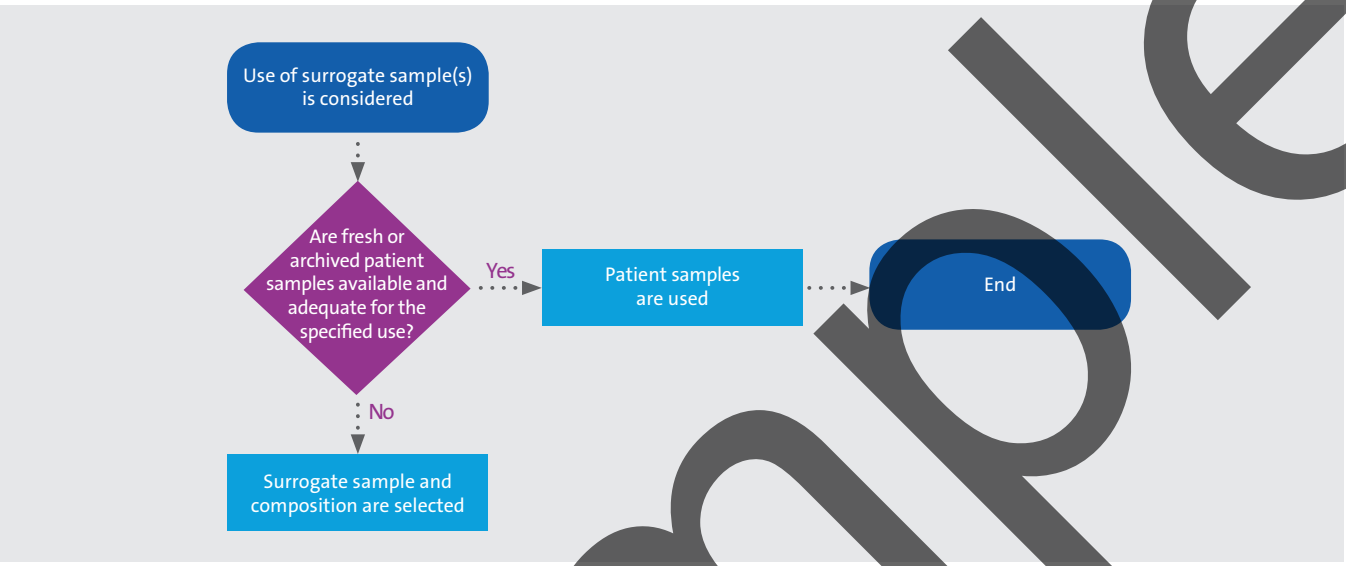
This guideline establishes a definition of surrogate sample: material or combination of materials used as a substitute for body fluid or tissue taken for examination from a single human subject to study the characteristic of interest. Physical separation, collection into a medical container, multiple collections from the same venipuncture draw, formalin fixation, or paraffin embedding **do not** confer surrogate sample designation. Surrogate analyte, surrogate matrix components, or a combination of surrogate analyte and matrix can be used in surrogate samples. Surrogate samples include but are not limited to:

- Pooled patient samples of biological origin
- Materials supplemented (eg, spiked) with an analyte of interest
- Material created to have properties similar to or representative of the body fluid or tissue of interest
- Material composed of a combination of an analyte that simulates the analyte of interest and a matrix created to have properties similar to or representative of the body fluid or tissue or of the patient or subject
- More-complex combinations of fabricated analyte and matrix

4 Surrogate Sample Hierarchical Approach

4.1 Decision to Use Surrogate Samples

Laboratories and commercial manufacturers (collectively "developers") prefer to use freshly collected, archived, or frozen patient samples for performance evaluations, some validations or verifications, and regulatory submissions. However, patient samples that are used to test the performance of an assay may be unavailable for several reasons. Figure 2 illustrates the process for deciding to use surrogate samples.



[a] Four basic symbols are used in this process flow chart: oval (signifies the beginning or end of a process), arrow (connects process activities), box (designates process activities), diamond (includes a question with alternative "Yes" and "No" responses).

Figure 2. Process for Deciding to Use a Surrogate Sample[a]

Depending on the test, the sample may be an unmodified specimen or a specimen that has been processed before examination (eg, use of chemical additives, extraction, centrifugation, or other physical separation methods), which are collectively known as "patient samples." In general, it is preferable to use surrogate samples to supplement testing conducted with patient samples, rather than relying solely on surrogate samples.

4.2 Surrogate Sample Hierarchy

After deciding to use surrogate samples, developers can apply a hierarchical approach to determine the appropriate surrogate sample type and combination for the designated use. The hierarchy shown in Table 3 minimizes the deviation between surrogate analyte and/or matrix and the patient samples used in the test. Table 3 depicts the possible combinations for surrogate samples and places them in a hierarchy, with pooled patient samples or supplemented individual patient samples at the top. This hierarchy serves as the starting point for composing surrogate samples and can be used, in conjunction with general principles and performance study–specific objectives, to select the best surrogate sample for the planned use or study. The developer should document the decisions and the rationale in a surrogate sample plan, as discussed in Chapter 5. For ease of use, samples in Table 3 have been assigned alphanumerical characters. Appendix A provides surrogate sample descriptions and examples.

5 Surrogate Sample Plan

Before using surrogate samples, developers should first consider the goals and objectives of each study or use and then determine whether and what type of surrogate samples are appropriate. A surrogate sample type that is suitable for one performance study or use may not be suitable for another study or use. Using the risk management principles described in international standards[13-15] and in CLSI documents EP18[17] and EP23,[16] the developer can create a surrogate sample plan based on performance study objectives, patient sample characteristics, the principles described in this guideline, and the surrogate sample hierarchy scheme. The plan is used to select the appropriate type and quantity of surrogate samples. It also serves as documentation of the scientific rationale and decision-making process. Figure 4 illustrates the process for developing a surrogate sample plan.

[a] Three basic symbols are used in this process flow chart: oval (signifies the beginning or end of a process), arrow (connects process activities), box (designates process activities).
[b] The analyte, matrix, volume, and concentration should mimic those of the patient sample.

Figure 4. Process for Developing a Surrogate Sample Plan[a]

Related CLSI Reference Materials[a]

C37 **Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures. 1st ed., 1999.** This guideline details procedures for the manufacture and evaluation of human serum pools for cholesterol measurement.

C49 **Analysis of Body Fluids in Clinical Chemistry. 2nd ed., 2018.** This guideline provides information for the medical laboratory for evaluating measurement procedures, as well as a strategy to characterize assay performance, when applied to body fluid matrixes. Key concepts that apply to the entire test cycle, including preexamination, examination, and postexamination phases of body fluid testing, are discussed.

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 Linearity of Quantitative Measurement Procedures. 2nd ed., 2020.** This guideline provides information for characterizing the linearity interval of a measurement procedure, validating a linearity interval claim (to be performed by the manufacturer), and verifying an established linearity interval claim (to be performed by the end user).

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 interferents on clinical chemistry test results.

EP09 **Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 3rd ed., 2018.** This guideline covers the design of measurement procedure comparison experiments using patient samples and subsequent data analysis techniques used to determine the bias between two *in vitro* diagnostic measurement procedures.

EP12 **User Protocol for Evaluation of Qualitative Test Performance. 2nd ed., 2008.** This document provides a consistent approach for protocol design and data analysis when evaluating qualitative diagnostic tests. Guidance is provided for both precision and method-comparison studies.

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.

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

Related CLSI Reference Materials (Continued)

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.

EP18 **Risk Management Techniques to Identify and Control Laboratory Error Sources. 2nd ed., 2009.** This guideline describes risk management techniques that will aid in identifying, understanding, and managing sources of failure (potential failure modes) and help to ensure correct results. Although intended primarily for *in vitro* diagnostics, this document will also serve as a reference for clinical laboratory managers and supervisors who wish to learn about risk management techniques and processes.

EP19 **A Framework for Using CLSI Documents to Evaluate Clinical Laboratory Measurement Procedures. 2nd ed., 2015.** This report uses the "measurement procedure lifecycle" framework to aid users of CLSI evaluation protocols documents during establishment and implementation of measurement procedures developed by both commercial manufacturers and clinical laboratories, ie, for laboratory-developed tests.

EP23™ **Laboratory Quality Control Based on Risk Management. 1st ed., 2011.** This document provides guidance based on risk management for laboratories to develop quality control plans tailored to the particular combination of measuring system, laboratory setting, and clinical application of the test.

EP25 **Evaluation of Stability of *In Vitro* Diagnostic Reagents. 1st ed., 2009.** This document provides guidance for establishing shelf-life and in-use stability claims for *in vitro* diagnostic reagents such as reagent kits, calibrators, and control products.

EP30 **Characterization and Qualification of Commutable Reference Materials for Laboratory Medicine. 1st ed., 2010.** This document provides information to help material manufacturers in the production and characterization of commutable reference materials, as well as to assist assay manufacturers and laboratorians in the appropriate use of these materials for calibration and trueness assessment of *in vitro* diagnostic medical devices.

EP34 **Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking. 1st ed., 2018.** 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.

EP35 **Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures. 1st ed., 2019.** This guideline provides recommendations for assessing clinically equivalent performance for additional similar-matrix specimen types and suitable performance for dissimilar-matrix specimen types, such that the laboratory does not necessarily need to repeat the full measurement procedure validation for each specimen type. The recommendations in this guideline apply to both quantitative measurement procedures and qualitative examinations.

Related CLSI Reference Materials (Continued)

EP37 **Supplemental Tables for Interference Testing in Clinical Chemistry. 1st ed., 2018.** This document includes recommended testing concentrations for analytes and endogenous substances that may interfere in clinical chemistry measurement procedures and is intended for use with the evaluation procedures in the Clinical and Laboratory Standards Institute guideline EP07.

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.

MM03 **Molecular Diagnostic Methods for Infectious Diseases. 3rd ed., 2015.** This report addresses topics relating to clinical applications, amplified and nonamplified nucleic acid methods, selection and qualification of nucleic acid sequences, establishment and evaluation of test performance characteristics, inhibitors, and interfering substances, controlling false-positive reactions, reporting and interpretation of results, quality assurance, regulatory issues, and recommendations for manufacturers and clinical laboratories.

MM06 **Quantitative Molecular Methods for Infectious Diseases. 2nd ed., 2010.** This document provides guidance for the development and use of quantitative molecular methods, such as nucleic acid probes and nucleic acid amplification techniques of the target sequences specific to particular microorganisms. It also presents recommendations for quality assurance, proficiency testing, and interpretation of results.

MM17 **Validation and Verification of Multiplex Nucleic Acid Assays. 2nd ed., 2018.** This guideline includes recommendations for analytical validation and verification of multiplex assays, as well as a review of different types of biological and synthetic reference materials.

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