CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

# C64

# Quantitative Measurement of Proteins and Peptides by Mass Spectrometry

This guideline describes the design, development, and validation of quantitative assays for proteins and peptides by mass spectrometry.

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

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## Abstract

Clinical and Laboratory Standards Institute guideline C64—*Quantitative Measurement of Proteins and Peptides by Mass Spectrometry* provides a framework for developing clinical protein and peptide assays from conception to validation. This guideline is intended for those who have experience with traditional small-molecule liquid chromatography–mass spectrometry (LC-MS) but not with protein and peptide analysis. Although closely related to traditional small-molecule analysis by LC-MS, protein and peptide analysis involves unique challenges and necessitates complex workflows, which are covered in detail. To enhance translation of assays to clinical use, this guideline focuses on method development aligned with clinically appropriate analytical validation.

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# Foreword

This guideline is intended to accompany CLSI documents C62[1] and EP19.[2] Many of the recommendations found in CLSI documents C62[1] and EP19[2] also apply to liquid chromatography–mass spectrometry (MS) protein measurements, and commonalities are highlighted. However, this guideline primarily concentrates on aspects that are unique to quantitative measurement of proteins and peptides by MS.

**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

**Assay development**
**Bioanalysis**
**Liquid chromatography**
**Mass spectrometry**
**Peptide**
**Protein**
**Surrogate peptide**
**Validation**

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# Chapter 1

# Introduction

## This chapter includes:

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

# Quantitative Measurement of Proteins and Peptides by Mass Spectrometry

## 1 Introduction

### 1.1 Scope

This guideline provides broad recommendations for appropriately developing and validating quantitative protein and peptide assays for clinical applications using electrospray liquid chromatography–mass spectrometry (LC-MS) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) techniques. C64 is practically focused and includes workflow overviews and experimental strategies for developing and validating quantitative assays for soluble proteins and peptides in biofluids (eg, serum, saliva, urine). It covers complex analyses, including measurement of proteins with post-translational modifications (PTMs). Although there are a diverse array of ionization modes and associated mass analyzers (eg, matrix-assisted laser desorption/ionization time-of-flight [MALDI-TOF] mass spectrometry [MS]), this guideline focuses on liquid chromatography (LC) and electrospray ionization (ESI) coupled with tandem mass spectrometry (MS/MS) because of the wide availability and proven utility of this method.

A protein or peptide associated with a medical diagnosis or clinical outcome may exist *in vivo* as a single specified molecular composition or as a complex collection of related proteoforms that differ in molecular composition. Given the heterogenous nature of proteins and the desirability of facilitating results standardization among different assays, the need to appropriately define the measurand is a key difference between small-molecule analysis and protein analysis. In order to design a suitable workflow for measurand assessment, the assay developer needs to consider analyte properties, enrichment and fractionation strategy, and instrument performance characteristics. Subsequently, calibrators and internal standards (IS) are selected based on the chosen workflow and a precisely defined measurand. At the beginning of method development, performance criteria guide the conception and refinement of the path of workflow. Following this iterative process, the developer eventually prepares a candidate method sufficiently robust to pass validation. Finally, rigorous validation studies are performed to demonstrate suitability for routine clinical use.

The intended users of this guideline are medical, research, and public health laboratories; *in vitro* diagnostic instrument manufacturers; and regulatory and accreditation organizations.

Tissues and other nonbiofluid specimens are not within this guideline's scope. Enzyme activity assays are also considered out of scope, as are detailed discussions of software tools and data processing algorithms used for *in silico* analysis of protein and peptide sequences.

### 1.2 Standard Precautions

Because it is often impossible to know what isolates or specimens 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 known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of bloodborne pathogens. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[3] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[4]