CLINICAL AND LABORATORY STANDARDS INSTITUTE®

3rd Edition

CLSI C62™

Liquid Chromatography–Mass Spectrometry Methods

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CLSI C62 provides guidance to laboratorians for reducing interlaboratory variance and evaluating interferences, assay performance, and other pertinent characteristics of liquid chromatography–mass spectrometry–based clinical assays. CLSI C62 emphasizes particular areas related to assay development and presents a standardized approach for method validation that is specific to mass spectrometry technology.

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

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Replaces CLSI C62-Ed2

Liquid Chromatography–Mass Spectrometry Methods

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Abstract

CLSI C62—*Liquid Chromatography–Mass Spectrometry Methods* provides guidance for the development and validation of liquid chromatography–mass spectrometry (LC-MS) methods in the medical laboratory. CLSI C62 is intended to support the development, validation, implementation, and maintenance of accurate and precise clinical assays through guidance for evaluating interferences, assay performance, and other pertinent characteristics specific to chromatographic and mass spectrometric techniques. CLSI C62 is intended for laboratorians responsible for the development and validation of mass spectrometry (MS)–based assays, health care providers who may use these assays for patient care decisions, external quality assessment programs, regulatory agencies, and manufacturers of MS instrumentation and reagent kits. CLSI C62 is limited to discussion of LC-MS and focuses on the steps for the development of a method, eg, whether the analyte is a drug, hormone, peptide, or protein.

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Foreword

CLSI C62 was developed in response to the need for increased robustness and harmonization of liquid chromatography–mass spectrometry (LC-MS) methods. Developers seeking to provide commercial kits and/or platforms using LC-MS have recognized the improvement in quality of analysis provided by the technology. For laboratories capable of developing and implementing laboratory-developed tests, mass spectrometry (MS) provides a flexible solution by enabling the rapid development of a robust measurement procedure or assay that meets detection capability and selectivity requirements. In some cases, MS offers an attractive alternative to commercially available assays that have already been developed using distinct technologies but do not offer acceptable performance in a given clinical context. LC-MS technology has also been used successfully to rapidly develop clinical assays for known and emerging biomarker research. LC-MS technology is routinely used in select areas of laboratory medicine and is an emerging technology in others. With ongoing advances in instrumentation, more widespread application of LC-MS technology in more routine clinical diagnostic testing is anticipated.

Of note, MS has become one of the key tools for the development of reference methods in laboratory medicine. There are a growing number of reference method procedures based on this technology. Despite the significant advantages that can be gained from incorporating LC-MS into the medical laboratory, a challenge of harmonization and standardization between laboratories using LC-MS still exists. One of these challenges related to standardization and the lack of commercially available matrix-appropriate calibrators is that each medical laboratory must often formulate its own calibrators. Some sites might use powdered or commercially lyophilized material to make calibrators, others might use organic solvent solutions, and still others might use formulations obtained from their institution's pharmacy. Moreover, the preparation of the calibrators also varies, from using solutions made in buffer, using analyte-free serum or plasma as the matrix, or using residual patient specimens as the calibrator matrix. Differences in chromatographic and mass spectrometric methods from site to site lead to variable matrix effects during analysis. Additionally, many laboratories validate and verify their assays using various protocols in accordance with different regulatory, accreditation, or industry standards. CLSI C62 covers these issues by providing guidance for the development, validation, and verification of LC-MS methods in the medical laboratory.

CLSI C62 outlines many important elements for the successful implementation of LC-MS technology for clinical methods. The basic instrument components needed for chromatography and MS are discussed, along with instrument parameters that must be optimized for the development of robust LC-MS methods. Additionally, a discussion of preexamination considerations that must be covered during the method development process is included. Various elements of method development are summarized, along with good practice recommendations for applying those elements during the process. Guidance is provided for validation and verification of an LC-MS method, including a recommendation for prevalidation evaluation before full method validation. Finally, guidance for QA, including assay QC and postimplementation monitoring, is provided.

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Overview of Changes

CLSI C62-Ed3 replaces CLSI C62-Ed2, published in 2022. Several changes were made in this edition, including:

- Calibration of an LC-MS test method
- Multiplexed quality controls
- Use of ion ratios
- Updates to development and validation recommended practices

NOTE: The content of CLSI C62 is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

chromatography

liquid chromatography–mass spectrometry

mass spectrometry

postimplementation monitoring

quality control

validation

verification

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Chapter 1

Introduction

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Next-Generation *In Vitro* Diagnostic Instrument Liquid Chromatography–Mass Spectrometry Methods

1 Introduction

1.1 Scope

CLSI C62 provides an introduction to and guidance for method development, validation, verification, and postimplementation monitoring of clinical applications using liquid chromatography–mass spectrometry (LC-MS), including the detection, diagnosis, and monitoring of diseases or disorders. Although LC-MS can also be used for untargeted qualitative analyses, the focus of CLSI C62 is on the use of this technology for targeted detection and/or quantification of clinically relevant analytes. Additionally, although there are commercial and research methods that enable direct injection without chromatography for rapid analyses, CLSI C62 is exclusively focused on LC-MS, including liquid chromatography–tandem mass spectrometry (LC-MS/MS). The purpose of CLSI C62 is to educate developers, clinical LC-MS practitioners, and health care providers who might use these test methods for patient care decisions on the benefits and limitations of LC-MS methods used in the medical laboratory, as well as provide a practical guide for the development and implementation of LC-MS–based clinical applications. It is intended to serve not only as a companion to CLSI C50,[1] which serves as general guidance for mass spectrometry (MS) in the medical laboratory, but also to provide an enhanced focus on methods, recommended practices, and instrumentation related to LC-MS. CLSI C62 is also intended as a resource for instrument manufacturers, manufacturers of LC-MS reagents, regulatory agencies, and educators, as well as individuals responsible for developing laboratory standards and policy. It serves as a foundational document for CLSI C64,[2] which provides specific guidance on quantitative protein MS methods.

A description of all current clinical applications of LC-MS, as well as pertinent information regarding development, validation, and verification of these methods, is beyond the scope of CLSI C62. As such, the reader is directed to appropriate existing resources wherever possible. In providing guidance for LC-MS method development, validation, and implementation, CLSI C62 focuses on:

- Important features of LC-MS instrumentation
- Preexamination factors that can affect assay performance and utility
- Assay calibration
- Analytical variables important in method development
- Assay validation and verification
- QA and QC
- Postimplementation method monitoring

The intended users of CLSI C62 are medical laboratorians, instrument manufacturers, regulatory agencies, health care providers, external quality assessment programs, and manufacturers of testing components or kits.

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

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