CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

C62

Liquid Chromatography-Mass Spectrometry Methods

This document provides guidance to the clinical laboratorian for the reduction of inter-laboratory variance and the evaluation of interferences, assay performance, and other pertinent characteristics of clinical assays. This guideline emphasizes particular areas related to assay development and presents a standardized approach for method verification 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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Clinical and Laboratory Standards Institute
P: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org

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Liquid Chromatography-Mass Spectrometry Methods

William Clarke, PhD, MBA, DABCC
Ross J. Molinaro, PhD, MLS(ASCP)^{CM}, DABCC, FACB
Lorin M. Bachmann, PhD, DABCC, MT(ASCP)
Julianne Cook Botelho, PhD
Zhimin Cao, MD, PhD, DABCC
Deborah French, PhD, DABCC
Seema Garg, MS, MBA
John M. Gawoski, MD
Russell P. Grant, PhD
Andrew N. Hoofnagle, MD, PhD
Bagyalakshmi Iyer, PhD
Vathany Kulasingam, PhD, FCACB
Donald S. Mason, MS
Brian Rappold
Danyel H. Tacker, PhD, DABCC
Steven M. Truscott, PhD, DABCC
Chunli Yu, MD, FACMG
Yusheng Zhu, PhD, DABCC, FACB

Abstract

Clinical and Laboratory Standards Institute guideline C62—*Liquid Chromatography-Mass Spectrometry Methods* provides guidance for the development and verification of liquid chromatography-mass spectrometry (LC-MS) methods in the clinical laboratory. The document is intended to reduce interlaboratory variance for clinical assays through guidance for evaluating interferences, assay performance, and other pertinent characteristics. It emphasizes particular areas related to assay development and presents a standardized approach for method verification that is specific to mass spectrometry (MS) technology. This document is intended for laboratorians responsible for development and verification of MS-based assays, physicians who may use these assays for patient care decisions, external quality assessment programs, and manufacturers of MS instrumentation and reagent kits designed to be paired with a particular mass spectrometer. This document is limited to discussion of LC-MS and is focused on the steps for development of a method, eg, whether the analyte is a drug, hormone, protein, or peptide.

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Document Development Committee on Liquid Chromatography/Mass Spectrometry Methods

William Clarke, PhD, MBA, DABCC
Chairholder
Johns Hopkins Medical Institutions
USA

Ross J. Molinaro, PhD, MLS(ASCP)^{CM}, DABCC, FACB
Vice-Chairholder
Siemens Healthcare Diagnostics, Inc.
USA

Lorin M. Bachmann, PhD, DABCC, MT(ASCP)
Virginia Commonwealth University
USA

Julianne Cook Botelho, PhD
Centers for Disease Control and Prevention
USA

Zhimin Cao, MD, PhD, DABCC
New York State Department of Health
USA

Russell P. Grant, PhD
LabCorp
USA

Andrew N. Hoofnagle, MD, PhD
University of Washington Medical Center
USA

Vathany Kulasingam, PhD, FCACB
University Health Network
Canada

Donald S. Mason, MS
Waters Corporation
USA

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Staff

Clinical and Laboratory Standards Institute
USA

Emily J. Gomez, MS, MLS(ASCP)^{CM}MB^{CM}
Project Manager

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Editor

Lisa M.W. Walker, MS, ELS
Editor

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Lorin M. Bachmann, PhD, DABCC, MT(ASCP)
Virginia Commonwealth University Health System
USA

William Clarke, PhD, MBA, DABCC
Johns Hopkins Medical Institutions
USA

Deborah French, PhD, DABCC
University of California San Francisco
USA

Seema Garg, MS, MBA
Eurofins Medinet
USA

John M. Gawoski, MD
Lahey Hospital & Medical Center
USA

Bagyalakshmi Iyer, PhD
Pennsylvania Department of Health
USA

Brian Rappold
Essential Testing, LLC
USA

Danyel H. Tacker, PhD, DABCC
West Virginia University Hospitals
USA

Steven M. Truscott, PhD, DABCC
Beaumont Health System
USA

Chunli Yu, MD, FACMG
Mount Sinai Genetic Testing Laboratory
USA

Yusheng Zhu, PhD, DABCC, FACB
Medical University Hospital Authority
USA

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Foreword

The importance of mass spectrometry (MS) in the clinical laboratory is increasing, and this CLSI document was developed in response to the need for increased robustness and harmonization of liquid chromatography-mass spectrometry (LC-MS) methods. Vendors of *in vitro* diagnostic devices often wait until the clinical utility of a particular assay is established and widely accepted before beginning development of a commercial assay. As a result, improvements in patient care can be delayed until a commercial assay is available. These delays can be significant if there are difficulties in the development of the assay. For laboratories with the capability to develop and implement laboratory-developed tests, MS provides an attractive alternative solution based on the ability to rapidly develop an analytically robust assay with excellent analytical sensitivity and specificity. In some cases, it offers an attractive alternative to commercially available assays that have already been developed, but do not offer acceptable performance in the current clinical context. LC-MS technology shows promise as a tool to rapidly develop clinical assays for emerging biomarkers coming from research in proteomics. Advances in instrumentation are likely to enable application of LC-MS technology in routine clinical diagnostic testing.

Despite the significant advantages that can be gained from incorporating MS into the clinical laboratory, considerable challenges exist. Among these is the fact that for some assays, significant laboratory-to-laboratory variability for the same analyte has been observed. There is currently limited assay standardization for MS-based methods, and much of the assay variability can be attributed to the lack of commercially available calibrators, that is, each clinical laboratory must formulate its own calibrators. For example, some sites may use powdered or commercially lyophilized material, some may use organic solvent solutions, and some may even use formulations obtained from their institution's pharmacy to make calibrators. Moreover, the preparation of the calibrator also varies from using solutions made in buffer, from using analyte-free serum or plasma as the matrix, to from using patient specimen remnants as the calibrator matrix—a problem for endogenous analytes. Differences in chromatographic methods from site to site lead to variable matrix effects during analysis. In addition, many laboratories verify their assays using various protocols in accordance with different regulatory or industry standards. This document addresses these issues by providing guidance for the development and verification of LC-MS methods in the clinical laboratory.

This document outlines many important elements for successful implementation of LC-MS technology for clinical analyses. The basic instrument components needed both for chromatography and MS are discussed, along with instrument parameters that must be optimized for development of robust LC-MS methods. In addition, the document contains a discussion of preexamination considerations that must be addressed during the method development process. Various elements of method development are summarized, along with best practice recommendations for addressing those elements during the process. Guidance is provided for verification of an LC-MS method, including a recommendation for prevalidation evaluation before full method verification. Finally, the document provides guidance for QA, including assay QC and postimplementation monitoring.

Overview of Changes

This guideline was revised in 2022 under the Limited Revision Process and replaces the first edition of the guideline, which was published in 2014. The sole change made in this edition is distinguishing recommended criteria for intralaboratory evaluation from studies required for regulatory submissions.

KEY WORDS

Chromatography

Liquid chromatography-mass spectrometry

Mass spectrometry

Method verification

Postimplementation monitoring

Quality control

Chapter 1

Scope

Liquid Chromatography-Mass Spectrometry Methods

1 Scope

This document provides an introduction to, and guidance for, method development, verification, and postimplementation monitoring of quantitative clinical applications using liquid chromatography-mass spectrometry (LC-MS). While LC-MS may also be used for qualitative analyses, the focus of this document is on the use of this technology for quantification of clinical analytes. In addition, while there are commercial and research methods that allow direct injection without chromatography for rapid analyses, this guideline is exclusively focused on liquid chromatography (LC) coupled to mass spectrometry (MS). The purpose of this guideline is to educate both clinical LC-MS practitioners and health care providers (including physicians) who may use these assays for patient care decisions on the benefits and limitations of LC-MS methods used in the clinical 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 document C50,[1] which serves as excellent general guidance for MS in the clinical laboratory, but also to provide an enhanced focus on methods, best practices, and instrumentation related to LC-MS, which is emerging as the most common approach to clinical analyses. This document is also intended to be 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.

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

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