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October 2007

C50-A

Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline

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.

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

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Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline

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Abstract

Clinical and Laboratory Standards Institute document C50-A—*Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline* provides a general understanding of MS 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 an 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. This document is intended to be a basic resource for clinical chemists; health practitioners; instrument manufacturers; and those responsible for developing standards, implementing policy, and teaching.

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Contents

Foreword

Since the early 1900s, mass spectrometry (MS) has been employed as an analytical tool in science (chemistry and physics) and industry (petroleum and pharmaceutical). Gas chromatography-mass spectrometry (GC-MS) was introduced into clinical medicine for the identification of inborn errors of organic acid metabolism; this type of MS analysis has been practiced for more than a quarter century.[1-3] Laboratories adopting this method were often specialized reference- or university-based medical centers, principally because of the complexity of sample preparation and the expertise required for interpretation of the results.

The development of more user-friendly, affordable, and versatile mass spectrometers has since allowed a large increase in the use of MS for clinical applications. This was also facilitated by the availability of stable isotopes that can serve as internal standards, thereby allowing for more accurate quantitation. An excellent history of MS has been published.[4] Most early clinical applications of MS employed GC-MS systems to analyze small biochemical compounds such as amino acids, fatty and organic acids, steroids, and simple carbohydrates; however, over the last two decades, rapid developments in ion sources have provided the opportunity to analyze more water-soluble, polar compounds, including peptides, proteins, oligonucleotides, DNA, and trace elements.[5]

The purpose of this document is to provide accurate and state-of-the-art information and guidance for the appropriate use of MS in the clinical laboratory. However, this document cannot cover all possibilities in this rapidly developing field, and the recommendations made herein should be interpreted in the light of continuing progress.

The greater part of this document focuses on a general understanding of MS and the principles that dictate its application in the clinical laboratory. To illustrate these concepts, portions of methods currently practiced and in widespread use are provided. More specific applications of some methods are provided in the appendix. This document is intended to be a basic resource for clinical chemists; health practitioners; instrument manufacturers; regulatory agencies; and those responsible for developing standards, implementing policy, and teaching. It is hoped that this document will be used to support future guidelines and recommendations for specific clinical applications pertaining to a mass spectrometric method.

A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. In light of this, CLSI recognizes that harmonization of terms facilitates the global application of standards and deserves immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In the context of this document, it is necessary to point out that the term *analyte* is used differently in the United States and other countries, notably those in Europe. ISO defines the term *analyte* as a component represented in the name of a measurable quantity; but uses the term *measurand* (a particular quantity subject to measurement) when the term relates to a biological fluid/matrix. In the United States, *analyte* is used to describe both a single component (analyte) as well as the analyte in its specific matrix (measurand).

Also, in order to align the usage of terminology in this document with that of ISO, the following terms are used in C50-A:

The term *accuracy,* in its metrological sense, refers to the closeness of the agreement between the result of a (single) measurement and a true value of a measurand, and comprises both random and systematic effects. *Trueness* is used in this document when referring to the "closeness of the agreement between the average value from a large series of measurements and a true value of a measurand"; the measurement of trueness is usually expressed in terms of *bias.* The term *measuring range* has replaced *reportable range* when referring to "a set of values of measurands (analytes) for which the error of a measuring instrument (test) is intended to lie within specified limits." The term *measurement procedure* has replaced *analytical method* when referring to a set of operations, described specifically, used in the performance of particular measurements according to a given method. The terms *diagnostic sensitivity* and *diagnostic specificity* have replaced the terms *clinical sensitivity* and *clinical specificity* because in Europe, the term "clinical" often refers to clinical studies of drugs under stringent conditions.

Users of C50-A should understand, however, that the fundamental meanings of the terms are identical in many cases, and to facilitate understanding, terms are defined in the Definitions section of this guideline.

All terms and definitions will be reviewed again for consistency with international use, and revised appropriately during the next scheduled revision of this document.

Key Words

Biomarker, GC-MS, ionization, isotope, LC-MS, mass spectra, mass spectrometry, mass spectrum, metabolism, proteins

Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline

1 Scope

This document provides an introduction to, and guidance, resources, and references for, the use of mass spectrometry (MS) in the clinical laboratory. It serves to illuminate specific issues in mass spectrometric analyses that must be considered when the technology is applied to clinical testing. This guideline aims to educate both the practitioners of MS and the medical professionals who use the results produced by the instruments for the diagnosis, characterization, or monitoring of disease. Through knowledge of this material, the medical professional will better understand why MS may be preferred for a clinical application. They will also become more informed consumers when selecting a diagnostic laboratory to provide MS services. This document is also intended to be a basic resource for instrument manufacturers; regulatory agencies; and those responsible for developing standards, implementing policy, and teaching.

Selected examples of "routinely utilized clinical assays" are used to describe the fundamental principles of MS. These examples are primarily from tests for small molecules and metabolites. There is also a brief discussion of the MS analysis of other analytes that are either not common in clinical chemistry application at the time of this writing or are highly specialized, warranting their own document. These analytes include elements, peptides, proteins, and other biopolymers, including oligonucleotides.

A description of all current clinical applications of MS is beyond the scope of this document. Therefore, the goal of this guideline is to provide a basic understanding of the technology and how it should be used in the clinical laboratory with an emphasis on:

- advantages and disadvantages;
- precautions required in its use;
- quality control awareness;
- assay verification/validation;
- approaches to reporting results; and
- communication of the data.

Portions of published and validated clinical methods are discussed in more detail to illustrate these concepts when required.

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 infectious agents and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention.[6] For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to CLSI document M29.[7]

3 Terminology

3.1 Definitions[a]

accuracy (of measurement) – closeness of agreement between the result of a measurement and a true value of the measurand (VIM93).[8]

analyte – component represented in the name of a measurable quantity (ISO 17511)[9]; **NOTE 1:** In the type of quantity "mass of protein in 24-hour urine," "protein" is the analyte. In "amount of substance of glucose in plasma," "glucose" is the analyte. In both cases, the long phrase represents the **measurand** (ISO 17511)[9]; **NOTE 2:** In the type of quantity "catalytic concentration of lactate dehydrogenase isoenzyme 1 in plasma," "lactate dehydrogenase isoenzyme 1" is the analyte (ISO 18153).[10]

analytical measurement range (AMR) – range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment, not part of the usual assay procedure.[11]

analytical run – a set of samples that are analyzed in a time period within which the measurement system is considered to have stable trueness and precision; **NOTE:** An analytical run usually consists of both quality control specimens and patient specimens.

bias – the difference between the expectation of the test results and an accepted reference value (ISO 3534-1).[12]

biomarker – a characteristic that is objectively measured and evaluated as an indicator of normal biologic or pathogenic processes or pharmacological responses to a therapeutic intervention; **NOTE:** In the context of this document, a biomarker is a chemical entity that can be quantified, and where there is a relationship between the amount of that entity and some pathogenic, pharmacologic, or therapeutic event.

biopolymers – a macromolecule in a living organism that is formed by linking together several smaller molecules (eg, DNA, RNA, polysaccharides, proteins, and peptides).

chemical ionization (CI) – an ionization process that leads to new ionized species arising from the interaction between molecules and gas phase ions, formed specifically for the purpose (ie, as "reagent ions"); **NOTE:** CI spectra are simpler than electron ionization (EI) spectra and are often dominated by an intense protonated or adducted molecular ion $(M+H)^+$ with few or no fragment ions.

clinical reportable range (CRR) – the range of analyte values that a method can report as a quantitative result, allowing for specimen dilution, concentration, or pretreatment used to extend the direct AMR.[13]

collision-induced dissociation (CID)//collisionally activated dissociation (CAD) – a process wherein a (fast) projectile ion is dissociated as a result of interaction with a target neutral species; **NOTE:** This is brought about by conversion, during the collision, of part of the ion's translational energy to internal energy.

dalton (Da) – see **unified atomic mass unit.**

[a] The mass spectrometry terms and definitions in this section are adopted from Sparkman OD. *Mass Spectrometry Desk Reference*. 1st ed. Pittsburgh, PA: Global View Publishing; 2000; and from Price P. Standard definitions of terms relating to mass spectrometry: a report from the Committee on Measurements and Standards of the American Society for Mass Spectrometry. *J Am Soc Mass Spectrom.* 1991;2:336-348.

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*. The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Customer Service
Personnel	Process Control	Assessments—External and Internal	Facilities & Safety

C50-A addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessments—External and Internal	Process Improvement	Customer Service	Facilities & Safety
GP26	GP26	GP26	X GP26	GP26 H3	C24, C43, EP5 EP6, EP9, EP10 EP15, EP17, GP26, GP27, H3, H4, H18, H56, LA4	GP26	GP26	EP10 GP26 GP27 GP29	GP26 GP27	GP26	GP26 H3

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*.

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

C50-A addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
H3 LA4	H3 H4 H56 LA4	H3 H18 H56 LA4	H3 H18 H56 LA4	H18 H56 LA4	X	X	H56	LA4

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*.

Related CLSI Reference Materials*

C24-A3 **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition (2006).** This guideline provides definitions of analytical intervals; planning of quality control procedures; and guidance for quality control applications.

C43-A **Gas Chromatography/Mass Spectrometry (GC/MS) Confirmation of Drugs; Approved Guideline (2002).** This document provides guidance for establishing uniform practices necessary for producing quality data for quantitation and identification of a drug or drug metabolite using the GC/MS method; specific quality assurance criteria for maintaining and documenting optimal instrument performance are also presented.

EP5-A2 **Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers' precision performance claims and determining when such comparisons are valid; as well as manufacturers' guidelines for establishing claims.

EP6-A **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (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.

EP9-A2 **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (2002).** This document addresses procedures for determining the bias between two clinical methods, and the design of a method comparison experiment using split patient samples and data analysis.

EP10-A3 **Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition (2006).** This guideline provides experimental design and data analysis for preliminary evaluation of the performance of a measurement procedure or device.

EP15-A2 **User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition (2005).** This document describes the demonstration of method precision and trueness for clinical laboratory quantitative methods utilizing a protocol designed to be completed within five working days or less.

EP17-A **Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004).** This document provides guidance for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of these limits.

GP26-A3 **Application of a Quality Management System Model for Laboratory Services; Approved Guideline—Third Edition (2004).** This guideline describes the clinical laboratory's path of workflow and provides information for laboratory operations that will assist the laboratory in improving its processes and meeting government and accreditation requirements.

GP27-A2 **Using Proficiency Testing to Improve the Clinical Laboratory; Approved Guideline—Second Edition (2007).** This guideline provides assistance to laboratories in using proficiency testing as a quality improvement tool.

GP29-A **Assessment of Laboratory Tests When Proficiency Testing is Not Available; Approved Guideline (2002).** This document offers methods to assess test performance when proficiency testing (PT) is not available; these methods include examples with statistical analyses. This document is intended for use by laboratory managers and testing personnel in traditional clinical laboratories as well as in point-of-care and bedside testing environments.

H3-A5 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fifth Edition (2003).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children. It also includes recommendations on order of draw.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

H4-A5 **Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Fifth Edition (2004).** This document provides a technique for the collection of diagnostic capillary blood specimens, including recommendations for collection sites and specimen handling and identification. Specifications for disposable devices used to collect, process, and transfer diagnostic capillary blood specimens are also included.

H18-A3 **Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Third Edition (2004).** This document includes criteria for preparing an optimal serum or plasma sample and for the devices used to process blood specimens.

H56-A **Body Fluid Analysis for Cellular Composition; Approved Guideline (2006).** This guideline provides users with recommendations for collection and transport of body fluids, numeration and identification of cellular components, and guidance for qualitative and quantitative assessment of body fluid.

LA4-A5 **Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard—Fifth Edition (2007).** This document addresses the issues associated with specimen collection, the filter paper collection device, and the application of blood to filter paper, and provides uniform techniques for collecting the best possible specimen for use in newborn screening programs.

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