CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

C57

Mass Spectrometry for Androgen and Estrogen Measurements in Serum

This guideline is intended to aid the laboratorian in developing appropriate procedures for the use of mass spectrometry in the measurement of androgens and estrogens.

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

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For additional information on committee participation or to submit comments, contact CLSI.

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Mass Spectrometry for Androgen and Estrogen Measurements in Serum

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Abstract

Clinical and Laboratory Standards Institute document C57—*Mass Spectrometry for Androgen and Estrogen Measurements in Serum* is intended to aid the laboratorian in developing appropriate procedures for the use of mass spectrometry (MS) in the measurement of androgens and estrogens. The primary objectives of this document are to provide guidance and establish uniform practices necessary for producing quality data for quantitation of androgens and estrogens. The guideline provides details specific to androgen and estrogen measurement procedures with respect to preexamination (preanalytical) considerations, MS technologies, measurement procedure and run validation, as well as postexamination (postanalytical) considerations.

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Contents

Contents (Continued)

Foreword

Androgen and estrogen measurements are widely used in clinical research, public health assessments, and patient care; however, problems that impede the translation of research and clinical findings into viable information for clinicians and scientists have been reported in the performance of these tests. As proposed by the Endocrine Society in a 2007 position statement[1] on measuring testosterone and concluded from the 2008 Centers for Disease Control and Prevention workshop[2] on steroid hormone testing, mass spectrometric procedures can overcome some of the current limitations in testing.

Mass spectrometry (MS) assays need to be developed and properly validated by the laboratory. This new technology, however, is not commonly used in the clinical laboratory and clinical chemists frequently are not familiar with developing these kinds of measurement procedures. As a result, the purpose of this document is to provide accurate, state-of-the-art information and guidance for the appropriate use of MS in the clinical laboratory for selected androgen and estrogen measurements in serum. Thus, this guideline may help in overcoming some of the current limitations in androgen and estrogen testing, and therefore aid in improving patient care and research translation.

Key Words

Androgen, estrogen, mass spectrometry, selected reaction monitoring, steroids

Mass Spectrometry for Androgen and Estrogen Measurements in Serum

1 Scope

This guideline describes principles, requirements, and recommendations of current mass spectrometry (MS) measurement procedures for routine analysis of androgens and estrogens in serum. The main focus of this document is on the analytical validation and clinical application of androgen and estrogen measurement procedures using MS. It includes guidance, references, and QA parameters that will assist with the implementation and operation of MS systems. Information on maintaining appropriate instrument settings and performance parameters, approaches to ensure accurate and precise measurements, measurement procedure validation requirements, QA procedures, and interpretation and reporting of results are included. Recommendations are included for sample preparation, and pre- and postexamination (pre- and postanalytical) considerations.

The intended users of this guideline are laboratorians who perform or plan to perform androgen and/or estrogen tests by MS, MS assay developers, and physicians and researchers involved in androgen and/or estrogen testing.

A general, comprehensive review of MS technologies in the clinical laboratory is provided in CLSI document C50.[3] This guideline is limited to the measurement of total androgens and/or estrogens in serum, referring to the free, bioavailable, albumin-bound androgens and estrogens, and free, bioavailable, sex hormone–binding globulin (SHBG)–bound androgens and estrogens. The focus of this guideline is limited to the measurement of androgens and estrogens commonly used in clinical and research settings that include, but are not limited to: dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAs), androstenedione, testosterone (T), dihydrotestosterone (DHT), estrone (E1), estrone sulfate (E1s), estradiol (E2), and estriol (E3). This guideline provides information on MS that relates to testing of the above-mentioned steroid hormones. In addition, the purpose of this document is to provide guidance on the appropriate use of MS for androgen and estrogen measurements and cannot cover all the possibilities in this rapidly developing field. The recommendations provided should be interpreted in light of the continuing progression in this discipline.

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. The Centers for Disease Control and Prevention address this topic in published guidelines that focus on the daily operations of diagnostic medicine in human and animal medicine while encouraging a culture of safety in the laboratory.[4] 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.[5]

3 Terminology

3.1 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, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI's consensus process for development and revision of standards and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

3.2 Definitions

accuracy (measurement) – closeness of agreement between a measured quantity value and a true quantity value of a measurand (JCGM 200:2012[6]).

analyte – component represented in the name of a measurable quantity (ISO 17511[7]); **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[7]); **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[8]).

bias – the difference between the expectation of the test results and an accepted reference value (ISO 5725-1[9]).

calibration – operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication (JCGM 200:2012[6]); **NOTE:** According to the US Code of Federal Regulations, calibration is the process of testing and adjusting an instrument or test system to establish a correlation between the measurement response and the value of the concentration or amount of the substance that is being measured by the test procedure (42 CFR 493.2[10]).

error (measurement)//measurement error – measured quantity value minus a reference quantity value (JCGM 200:2012[6]).

imprecision – dispersion of independent results of measurements obtained under specified conditions; **NOTE:** It is expressed numerically as standard deviation or coefficient of variation.

limit of quantitation (LoQ) – lowest amount of a measurand in a material that can be quantitatively determined with stated accuracy (as total error or as independent requirements for bias and precision), under stated experimental conditions (modified from ISO 18113-1[11]).

lower limit of the measuring interval (LLMI) – the lowest measurand concentration at which all defined performance characteristics of the measurement procedure are met; **NOTE:** Formerly, the term "lower limit of quantitation" was used in CLSI documents.

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system (QMS) 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 QMS 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 QSEs are as follows:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

C57 addresses the QSE 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 page 50.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
					C50	X EP05 EP06 EP07 EP09 EP10 EP14 EP15 EP17 EP23 EP28 EP30 EP32 GP44				EP10	
QMS01	QMS01	M29 QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01 QMS06

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

C57 addresses the clinical laboratory path of workflow process 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
QMS01	GP44 QMS01	GP44 QMS01	X GP44 QMS01	EP23 QMS01	C50 EP23 QMS01	C50 EP14 EP23 QMS01	QMS01	QMS01

Related CLSI Reference Materials*

C50 **Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance. 1st ed., 2007.** 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 CLSI-IFCC joint project.

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 the Linearity of Quantitative Measurement Procedures: A Statistical Approach. 1st ed., 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.

EP07 **Interference Testing in Clinical Chemistry. 2nd ed., 2005.** This document provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interfering substances on clinical chemistry test results.

EP09 **Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 3rd ed., 2013.** This document addresses 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.

EP10 **Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures. 3rd ed., 2014.** This guideline provides experimental design and data analysis for preliminary evaluation of the performance of a measurement procedure or device.

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.

EP15 **User Verification of Precision and Estimation of Bias. 3rd ed., 2014.** This document describes the estimation of imprecision and of bias for clinical laboratory quantitative measurement procedures using a protocol that can be completed within as few as five days.

EP17 **Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. 2nd ed., 2012.** This document provides guidance for evaluation and documentation of the detection capabilities 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.

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.

EP28 **Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory. 3rd ed., 2010.** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests. A CLSI-IFCC joint project.

* 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)

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 assist assay manufacturers and laboratorians in the appropriate use of these materials for calibration and trueness assessment of *in vitro* diagnostic medical devices.

EP32 **Metrological Traceability and Its Implementation. 1st ed., 2006.** This document provides guidance to manufacturers for establishing and reporting metrological traceability.

GP44 **Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests. 4th ed., 2010.** This document includes criteria for preparing an optimal serum or plasma sample and for the devices used to process blood specimens.

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.

QMS01 **Quality Management System: A Model for Laboratory Services. 4th ed., 2011.** This document provides a model for medical laboratories that will assist with implementation and maintenance of an effective quality management system.

QMS06 **Quality Management System: Continual Improvement. 3rd ed., 2011.** This guideline considers continual improvement as an ongoing, systematic effort that is an essential component of a quality management system. A continual improvement program may consist of fundamental processes and common supporting elements described in this guideline.

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