



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.

# Clinical and Laboratory Standards Institute

*Setting the standard for quality in clinical laboratory testing around the world.*

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing clinical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

## Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement, but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

## Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advancements in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential, and may be submitted by anyone, at any time, on any document. All comments are addressed according to the consensus process by a committee of experts.

## Appeals Process

If it is believed that an objection has not been adequately addressed, the process for appeals is documented in the CLSI Standards Development Policies and Process document.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

## Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

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

Clinical and Laboratory Standards Institute  
950 West Valley Road, Suite 2500  
Wayne, PA 19087 USA  
P: 610.688.0100  
F: 610.688.0700  
[www.clsi.org](http://www.clsi.org)  
[standard@clsi.org](mailto:standard@clsi.org)

C57, 1st ed.  
February 2015

---

## Mass Spectrometry for Androgen and Estrogen Measurements in Serum

Julianne Cook Botelho, PhD  
Lorin M. Bachmann, PhD, DABCC  
Zhimin Cao, MD, PhD, DABCC  
Donald Walt Chandler, PhD  
Nigel Clarke, PhD  
Laurence M. Demers, PhD  
Robert L. Fitzgerald, PhD, DABCC  
M. P. George, MS

Lisa M. Sapp, MS, MBA  
Patrick M. Sluss, PhD, MA  
Samuel A. Testino, Jr., PhD  
Christina Wang, MD  
Randy A. Weintraub, MS, PhD  
Regina G. Ziegler, PhD, MPH  
Hubert W. Vesper, PhD

### 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.

Clinical and Laboratory Standards Institute (CLSI). *Mass Spectrometry for Androgen and Estrogen Measurements in Serum*. 1st ed. CLSI guideline C57 (ISBN 1-56238-995-5 [Print]; ISBN 1-56238-996-3 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2015.

The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at [www.clsi.org](http://www.clsi.org). If you or your organization is not a member and would like to become one, and to request a copy of the catalog, contact us at: Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: [customerservice@clsi.org](mailto:customerservice@clsi.org); Website: [www.clsi.org](http://www.clsi.org).



C57, 1st ed.

Copyright ©2015. Clinical and Laboratory Standards Institute. Except as stated below, any reproduction of content from a CLSI copyrighted standard, guideline, companion product, or other material requires express written consent from CLSI. All rights reserved. Interested parties may send permission requests to [permissions@clsi.org](mailto:permissions@clsi.org).

CLSI hereby grants permission to each individual member or purchaser to make a single reproduction of this publication for use in its laboratory procedure manual at a single site. To request permission to use this publication in any other manner, e-mail [permissions@clsi.org](mailto:permissions@clsi.org).

### **Suggested Citation**

CLSI. *Mass Spectrometry for Androgen and Estrogen Measurements in Serum*. 1st ed. CLSI guideline C57. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

ISBN 1-56238-995-5 (Print)  
ISBN 1-56238-996-3 (Electronic)  
ISSN 1558-6502 (Print)  
ISSN 2162-2914 (Electronic)

Volume 35, Number 4

## Committee Membership

### Consensus Committee on Clinical Chemistry and Toxicology

**David G. Grenache, PhD,**  
**DABCC, FACB**  
**Chairholder**  
**University of Utah and**  
**ARUP Laboratories**  
**USA**

**Loralie J. Langman, PhD**  
**Vice-Chairholder**  
**Mayo Clinic**  
**USA**

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

Johanna Camara, PhD  
National Institute of Standards and  
Technology  
USA

Yung W. Chan, MT(ASCP)  
FDA Center for Devices and  
Radiological Health  
USA

Ann M. Gronowski, PhD  
Washington University School of  
Medicine  
USA

T. Scott Isbell, PhD, DABCC,  
FACB  
St. Louis University-Laboratory  
USA

Gregory T. Maine, PhD, FACB  
Abbott  
USA

Joseph Passarelli  
Roche Diagnostics Corporation  
USA

T. Andrew Quintenz  
Bio-Rad Laboratories, Inc.  
USA

### Document Development Committee on Mass Spectrometry for Androgen and Estrogen Measurements in Serum

**Julianne Cook Botelho, PhD**  
**Co-Chairholder**  
**Centers for Disease Control and**  
**Prevention**  
**USA**

**Lorin M. Bachmann, PhD,**  
**DABCC**  
**Co-Chairholder**  
**Virginia Commonwealth**  
**University**  
**USA**

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

Laurence M. Demers, PhD  
Penn State Hershey  
Medical Center  
USA

Robert L. Fitzgerald, PhD, DABCC  
University of California San Diego  
USA

Patrick M. Sluss, PhD, MA  
Massachusetts General Hospital  
USA

Samuel A. Testino, Jr., PhD  
Cryolife, Inc.  
USA

Christina Wang, MD  
Harbor-UCLA Medical Center  
USA

Randy A. Weintraub, MS, PhD  
Smithers Visient Laboratories  
USA

Regina G. Ziegler, PhD, MPH  
National Cancer Institute  
USA

#### Staff

Clinical and Laboratory Standards  
Institute  
USA

Luann Ochs, MS  
*Senior Vice President – Operations*

Ron S. Quicho, MS  
*Project Manager*

Megan L. Tertel, MA  
*Editorial Manager*

Joanne P. Christopher, MA  
*Editor*

Patrice E. Polgar  
*Editor*

C57, 1st ed.

### **Acknowledgment**

CLSI, the Consensus Committee on Clinical Chemistry and Toxicology, and the Document Development Committee on Mass Spectrometry for Androgen and Estrogen Measurements in Serum gratefully acknowledge the following volunteers for their important contributions to the development of this document:

Donald Walt Chandler, PhD  
Endocrine Sciences  
USA

M. P. George, MS  
Alere Toxicology Services  
USA

Hubert W. Vesper, PhD  
Centers for Disease Control  
and Prevention  
USA

Nigel Clarke, PhD  
Nichols Institute,  
Quest Diagnostics  
USA

Lisa M. Sapp, MS, MBA  
AB SCIEX  
USA

## Contents

Abstract.....	i
Committee Membership.....	iii
Foreword.....	vii
1 Scope.....	1
2 Standard Precautions.....	1
3 Terminology.....	2
3.1 A Note on Terminology.....	2
3.2 Definitions.....	2
3.3 Abbreviations and Acronyms.....	4
4 Preexamination (Preanalytical) Considerations.....	5
4.1 Specimen Requirements.....	5
4.2 Collection, Processing, and Storage.....	5
4.3 Preparation of Assay Calibration Materials and Quality Control Samples.....	6
4.4 Sample Preparation and Derivatization.....	8
5 Mass Spectrometry Technologies.....	13
5.1 Gas Chromatography Mass Spectrometry, Liquid Chromatography Mass Spectrometry, Tandem Mass Spectrometry.....	13
5.2 Measurement Procedure Characteristics for Steroid Hormone Measurement by Liquid Chromatography Tandem Mass Spectrometry.....	18
6 Measurement Procedure Validation/Characterization – Analytical Parameters.....	21
6.1 Accuracy/Bias.....	22
6.2 Specificity/Selectivity of the Process.....	22
6.3 Carryover.....	23
6.4 Imprecision.....	23
6.5 Linearity.....	24
6.6 Sensitivity.....	24
6.7 Measurement Uncertainty.....	24
7 Result Evaluation and Reporting.....	24
7.1 Assay Setup.....	24
7.2 Data Collection.....	26
7.3 Use of Confirmation Ions.....	28
7.4 Quality Control Monitoring.....	28
8 Quality Assurance Procedures.....	29
8.1 Staff Training and Competency Assessment.....	29
8.2 Facility and Resource Management.....	29
8.3 Policies and Procedures.....	30
8.4 Quality Assessment and Improvement.....	30
8.5 Proficiency Testing.....	31
9 Harmonization of Results.....	31

C57, 1st ed.

## Contents (Continued)

10	Postexamination (Postanalytical) Considerations .....	33
10.1	Reference Intervals .....	33
10.2	Data Evaluation and Reporting .....	34
	References .....	35
	Appendix A. Examples of Published Transitions for Selected Reaction Monitoring of Nonderivatized Steroid Hormones by Liquid Chromatography Tandem Mass Spectrometry .....	40
	Appendix B. Androgens, Estrogens, and Analogs .....	43
	Appendix C. Checklist for Run Evaluation .....	45
	Appendix D. Metrological Institutions .....	46
	The Quality Management System Approach .....	48
	Related CLSI Reference Materials .....	50



## **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<sup>1</sup> on measuring testosterone and concluded from the 2008 Centers for Disease Control and Prevention workshop<sup>2</sup> 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

This is a preview of "CLSI C57-Ed1". [Click here to purchase the full version from the ANSI store.](#)

C57, 1st ed.

## 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.<sup>3</sup> 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.<sup>4</sup> 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.<sup>5</sup>