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1st Edition

CLSI 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.

C57, 1st ed.
February 2015

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.

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.

C57, 1st ed.

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.

Reaffirmed:
August 2020

Archived:
May 2025

ISBN 1-56238-995-5 (Print)
ISBN 1-56238-996-3 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic) Volume 35, Number 4

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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.

Appendix A. Examples of Published Transitions for Selected Reaction Monitoring of Nonderivatized Steroid Hormones by Liquid Chromatography Tandem Mass Spectrometry

Steroid Hormone	Ion	Mass Transition	Ionization Source	# of Steroids	LoQ (ng/L)	Analytical Column	Sample Preparation	Reference
Androstenedione	$[M+H]^+$	287/97	ESI	10	404	C18	PP	1
		287/109; 97	APCI	3	100	C18	PP+SPEOL	2
		287/97	ESI	3	30	C18	SPE	3
		287/97	ESI	2	72	C18	LLE	4
		287/97; 109	APCI	9	20	C18	PP+SPE	5
DHEA*	$[M-H2O+H]^+$	271/197; 213	APCI	9	20	C18	PP+SPE	5
DHEAs	$[M-H]^-$	367/97	ESI	1	$3.6e^5$	C18	PP	6
Testosterone	$[M+H]^+$	289/97; 109	ESI	2	20	C18	LLE	7, 8
		289/97	ESI	1	50	C18	LLE	9
		289/97; 109	APCI	1	100	C18	PP+SPEOL	10
		289/109	ESI	10	600	C18	PP	1
		287/109; 97	APCI	3	100	C18	PP+SPEOL	2
		287/109; 97	APCI	1	3	C12	SPEOL	11
		289/97	ESI	1	61	C18	PP+LLE	12
		289/97	ESI	1	87	C18	PP	13
		289/97	ESI	3	30	C18	SPE	3
		289/109	ESI	2	72	C18	LLE	4
		289/109; 97	APCI	9	20	C18	PP+SPE	5
		289/97; 109	ESI	1	10	C18	LLE	14
		289/97	APCI	8	20	C18	LLE	15
		289/97; 109	ESI	1	20	C18	LLE	16
DHT	$[M+H]^+$	291/255	ESI	2	20	C18	LLE	7, 8
		291/255	ESI	10	854	C18	PP	1
		291/255	ESI	3	30	C18	SPE	3
Estradiol	$[M-H]^-$	271/145; 183	ESI	4	2†	C8	PP	17
Estrone	$[M-H]^-$	269/145; 143	ESI	4	1†	C8	PP	17
Estrone-sulfate	$[M-SO_3^-]^-$	349/269	ESI	1	8	C18	PP	18
		349/269	ESI	1	80	C18	SPE	19

* Water-loss ion.
† Value stated as "LOD" with CV < 10%.

Abbreviations: APCI, atmospheric pressure chemical ionization; CV, coefficient of variation; DHEA, dehydroepiandrosterone; DHEAs, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; ESI, electrospray ionization; LLE, liquid-liquid extraction; LoD, limit of detection; LoQ, limit of quantitation; PP, protein precipitation; SPE, solid phase extraction; SPEOL, solid phase extraction online.

NOTE: The *m/z* of the tenth decimal position may vary slightly, based on instrument tuning. Therefore, values for *m/z* are given to the nearest whole number.

Adapted from *Clinical Biochemistry*, Vol 44; Kushnir MM, Rockwood AL, Roberts WL, Yue B, Bergquist J, Meikle AW. Liquid chromatography tandem mass spectrometry for analysis of steroids in clinical laboratories; 77-88. © 2011, with permission from Elsevier.[20]

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PRINT ISBN 1-56238-995-5

ELECTRONIC ISBN 1-56238-996-3

CLSI C57-Ed1