Laboratory Guidelines

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Quantification of Endogenous Steroids in Blood for the *Athlete Biological Passport*

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1.0 Objective

These Laboratory Guidelines have been developed to ensure a harmonized application of the Analytical *Testing* Procedure**Analytical and Reporting Requirements for the** quantification of endogenous steroid**Blood *Markers*** measured in**of the Steroidal Module of the *Athlete Biological Passport***

1.0 Introduction

The purpose of this *Technical Document* (*TD*)**,** which constitutes an integral part of the *International Standard* for Laboratories (ISL) [1], is to harmonize the analysis and reporting of the blood (serum) **as part***Markers* of the Steroidal Module of the *Athlete Biological Passport* (*ABP*)**. The document provides guidance on the pre-analytical details, *Sample* preparation procedure, the performance of the analyses and the reporting of the test results**) to uncover the *Use* of synthetic forms of Endogenous Anabolic Androgenic Steroids (EAAS), in particular testosterone and its precursors.

2.0 Scope

These Laboratory Guidelines contain requirements for the implementation of the Analytical *Testing* Procedure for the quantification of endogenous *steroid Markers* in blood (serum) as part of the Steroidal Module of the *ABP* to uncover use of endogenous anabolic androgenic steroids (EAAS) administered exogenously. These Laboratory Guidelines follow the rules established in the *WADA International Standard for* Laboratories (ISL) [1] and relevant *Technical Documents* (*TDs*) regarding the Analytical *Testing* of blood *Samples*.

3.0 Introduction to the Analytical *Testing* Procedure

The Analytical *Testing* Procedure involves the measurement of two (2) *Markers*, namely Testosterone (T) and Androstenedione (Androst-4-ene-3,17-dione, A4), which are naturally present in blood, and the calculation of the T/A4 ratio. While the endogenous levels of these *Markers* are gender-specific, they have been identified as relevant target Analytes to detect T abuse with an increased sensitivity in female *Athletes* [2,3], as well as the transdermal application of T-related drugs in both genders [4–6].

1.1 The quantification of T and A4 concentrations is based on **Procedure for Analysis of the Blood Steroid *Markers***

The Analytical *Testing* Procedure (ATP) involves the measurement of the serum concentrations of two (2) naturally occurring EAAS, namely Testosterone (T) and Androstenedione (Androst-4-ene-3,17-dione, A4), and the automatic calculation of the T/A4 ratio in *ADAMS*.

a) The ATP for the blood steroid *Markers* is not a mandatory ATP (see ISL *TD* ATP [2]) and is not applied to all serum *Samples*. Therefore, the blood steroid *Markers* shall be measured in serum *Sample*(s) (see Article 2.0) by Laboratories with appropriate analytical capacity and upon request by the *Testing* Authority (TA) or *WADA*, and results shall be reported in *ADAMS*.

b) The analysis of the blood steroid *Markers* follows a two (2)-step procedure:

i. An Initial *Testing* Procedure (ITP) based on the quantification of the concentrations of the blood steroid *Markers* by Liquid Chromatography **(LC)** combined with **tandem** Mass

Spectrometry (LC-MS^n~~; n ≥ 1). For the purposes of the *ABP*, an initial quantification from the "A" *Sample* is performed. When requested, a confirmatory quantification of the "A" *Sample* may additionally be performed (see Article 6.2) to confirm the concentrations and to perform~~) (see Article 3.2), and

~~i.~~ii. A subsequent Confirmation Procedure (CP) may be performed, which consists of the LC-MS^n quantification and identification ~~of the *Markers* (as per TD IDCR[7]).~~ISL *TD* IDCR [3]) of the blood steroid *Markers* (see Article 4.0). A CP shall be performed when at least one primary blood steroid *Marker* (T or T/A4) in the *Sample* constitutes an outlier in the corresponding Passport for elevated values, as determined by the Adaptive Model, triggering an *Atypical Passport Finding* – Confirmation Procedure Request (*ATPF*-CPR) in *ADAMS*. A CP may also be performed upon request to the Laboratory (see Article 4.2).

~~The concentrations of T and A4 in blood reported by the Laboratories are integrated in the Steroidal Module of *ADAMS*, using a similar Bayesian approach to that applied in the other Steroidal (urine), Hematological and Endocrine Modules of the *ABP*.~~

~~1.0~~2.0 Assay Pre-analytical Procedure

a) The Laboratory should (usually) receive refrigerated (not frozen[i]) "A" and "B" blood *Samples*, which have been collected in blood "serum tubes" containing an inert polymeric serum separator gel and a clotting activation factor (for example: BD Vacutainer® SST™-II Plus tubes, EU ref 367955; BD Vacutainer® SST™-II Plus Advance tubes, EU ref 367954; BD Vacutainer® SST™ tubes, US ref 367986) in accordance with the *International Standard* for *Testing* ~~and Investigation (ISTI)~~ [7]~~;~~ (*IST*) [4]. The use of alternative collection devices shall be validated by the relevant Laboratory(ies) and approved by *WADA* prior to use for *Sample* collection.

b) Alternatively, if the clotting and centrifugation of the blood *Sample* is performed prior to reception at the Laboratory (for example, at the site of *Sample* collection~~),~~) or when a blood *Sample* is shipped from another Laboratory for subcontracted analyses, *Samples* may be received at the Laboratory as frozen/refrigerated ~~blood~~ *Samples* either in the same *Sample* collection tubes or as separated serum in new tubes~~;~~.

c) The Laboratory shall check the status of the *Sample*(s~~) (*e.g.*, evidence of hemolysis~~) and the integrity of the collection tubes (*e.g.,* evidence of breakage of the separating gel). The Laboratory shall note any unusual condition of the *Sample* and record such condition(s) in the ~~Test Report~~Lab Results in *ADAMS*~~;~~.

d) Any *Samples* delivered to the Laboratory in tubes containing an anti-coagulant (for example, ~~*ABP*~~whole blood *Samples* collected in EDTA tubes), or as separated plasma, shall not be analyzed for the blood steroid *Markers* ~~of the Endocrine Module;~~.

e) The Laboratory shall notify and seek advice from the *Testing* Authority (TA) regarding rejection or Analytical *Testing* of *Samples* for which irregularities are noted (see ISL ~~4)~~ [1]~~.~~).

[i] ~~unless~~Unless the blood matrix components have been separated before shipment to the Laboratory.

1.1.2.1. Blood *Samples* received Received as non Non-separated blood in tubes containing an inert polymeric serum separator gel and a clotting activation factor: "Serum Tubes"

Reception	Both *Samples* "A" and "B" shall be centrifuged for 10-15 min at 1300-1500 g as soon as possible after reception at the Laboratory. The "A" *Sample* shall be used for the initial and confirmatory (if needed) quantifications (see below). The "B" *Sample* shall be step-frozen and stored until use, if needed (see below).
Aliquoting and analysis *Sample* Processing upon Reception	– Both "A" and "B" *Samples* shall be centrifuged for 10-15 min at 1300-1500 g as soon as possible after reception at the Laboratory. "A" *Sample* An Aliquot of the "A" *Sample* serum shall be taken for initial quantification. – The remaining "A" serum fraction If the "A" *Sample* is not opened to be analyzed within five (5) days from *Sample* collection, then the Laboratory may be kept : • Keep the centrifuged "A" *Sample* in the *Sample* collection tube or aliquoted and step-freeze it (at approx. -15°C or less and according to the tube manufacturer's instructions) until thawing and aliquoting for analysis, or • Aliquot the separated serum fraction into new vials with label(s) (ensuring that appropriate Laboratory Internal Chain of Custody (see ISL *TD* LCOC) [5] is maintained.), which shall be stored frozen (at approx. -15°C or less) until thawing for analysis. For initial quantification: – the Aliquot may be analyzed immediately after aliquoting; or – the Aliquot shall be stored at approximately 4 °C°C if analyzed within 24h (within a maximum of five (5) days from *Sample* collection); or – the Aliquot shall be frozen (-20°C) if the analysis will be conducted more than 24h after aliquoting. – For the confirmatory quantification, a new Aliquot of the "B" *Sample* The centrifuged "B" *Sample* shall be step-frozen and stored (at approx. -15°C or less and according to the tube manufacturer's instructions) until use, if needed (see below). *[Comment: If the Laboratory transfers the Aliquot into new vials for frozen storage, the vials should ensure proper sealing for optimal storage (cryovials with an "O-ring"). Thawing of Sample(s) for analysis should be done stepwise; Samples shall not be thawed under hot water or any other similar process that risks raising the temperature of the Sample above room temperature. Thawing overnight under refrigeration (2-8 °C) is recommended.]* "A" *Sample* shall be analyzed immediately after aliquoting. *[Comment: When analyses specific to the ABP are requested for blood (serum) Samples (i.e., Markers of the Endocrine Module or blood steroid Markers as part of the Steroidal Module), only the "A" Sample should be considered for the initial and the confirmatory quantifications of the Markers. In cases where the "A" Sample is not suitable for the performance of ABP Markers quantification (e.g., there is insufficient Sample volume; the Sample container has not been properly sealed or has been broken; the Sample's integrity has been compromised in any way; the "A" Sample is missing), a splitting procedure of the "B" Sample could be performed, as detailed in the ISL[4].]*
Storage [The same storage conditions apply for *Samples* received in conditions described in section	Storage for up to three (3) months → at approximately -20 °C. Storage for more than three (3) months → freeze at approximately -20 °C and transfer to approximately -70 to -80 °C. a) *[Comment: If* ITP

~~4.2]~~*Sample* Processing for Analysis	An Aliquot of the ~~*separated*~~"A" *Sample* serum fraction ~~*is*~~shall be taken for the ITP of the blood steroid *Markers*, and shall be processed as follows: • It may be analyzed immediately after aliquoting; or • It may be stored refrigerated (2-8 °C) if analyzed within a maximum of five (5) days from *Sample* collection; or • It shall be stored frozen (at approx. -15 °C or less) if the analysis will be conducted more than five (5) days from *Sample* collection. The remaining "A" serum fraction may be kept in the *Sample* collection tube~~*, it shall be step-frozen for storage according to the tube manufacturer's instructions until analysis.*~~ ~~*If the Laboratory transfers the Aliquot*~~ or aliquoted into new ~~*vials for*~~vial(s) and shall be stored frozen ~~*storage,*~~(at approx. -15 °C or less) if the ~~*vials should ensure proper sealing for optimal storage (cryovials with an "O-ring").*~~ ~~*Thawing of Sample(s) for*~~ analysis ~~*should also be done stepwise. Samples shall not be thawed under hot water or any other similar process that risks raising the temperature of the Sample above room temperature. Thawing overnight at 4°C is recommended.]*~~will be conducted more than five (5) days from *Sample* collection[ii]. b) CP The CP shall be performed on a new Aliquot of the remaining "A" *Sample* serum fraction and shall be conducted immediately after aliquoting.

~~1.2~~2.2 Blood *Samples* ~~received~~Received as ~~frozen/refrigerated centrifuged blood/serum *Samples*:~~Centrifuged and Frozen/Refrigerated

Sample Processing upon Reception	a) If *Samples* are received frozen, they ~~should~~shall remain frozen until thawing and aliquoting for analysis~~ as described in this Article 4.2~~. b) If *Samples* are received refrigerated, ~~they~~the "A" *Sample* should be processed ~~as~~ to obtain an Aliquot for analysis as soon as possible (as per Article 42.1~~.~~.), while the "B" *Sample* shall be stored frozen (at approx. -15 °C or less) until aliquoting for analysis.
~~Aliquoting~~Aliquot, Storage and ~~analysis~~Analysis	a) ITP i. Once a serum Aliquot of the "A" *Sample* ~~"A"~~ is ~~thawed, an Aliquot shall be~~ taken for ~~initial quantification. This Aliquot~~ the ITP of the blood steroid *Markers*, it should: • Be analyzed immediately or may be stored ~~at approximately 4~~refrigerated (2-8 °C~~ for~~) if analyzed within a maximum of ~~24h before~~ five (5) days from *Sample* collection; or • Stored frozen (at approx. -15 °C or less) if the analysis~~.~~ is to be conducted after five (5) days from *Sample* collection [3]. ~~i.~~ii. The remaining "A" serum fraction ~~may be kept in the *Sample* collection tube or aliquoted into new vial(s) with label(s) ensuring Laboratory Internal Chain of Custody is maintained.~~ shall be stored as per Article 2.1 above. b) ~~For the confirmatory quantification,~~CP

[ii] It is recommended that the Laboratory stores the serum *Samples* frozen (at approx. -70 °C or less) if the TA (or *WADA*) has requested the Laboratory to place them into long-term storage (> 3 months) for Further Analysis purposes (see also ISL Article 5.3.7.2).

	The CP shall be performed on a new Aliquot of the remaining "A" *Sample* serum fraction and shall be ~~analyzed~~conducted immediately after aliquoting.

~~4.0 Analytical *Testing* Procedure Requirements~~

[Comment to Articles 2.1 and 2.2: When analyses specific to the ABP are requested, only the "A" Sample shall be considered for the ITP and CP. In cases where the "A" Sample is not suitable for the performance of the ABP Markers analysis (e.g., there is insufficient Sample volume; the Sample container has not been properly sealed or has been broken; the Sample's integrity has been compromised in any way; the "A" Sample is missing), a splitting procedure of the "B" Sample could be performed, as detailed in the ISL [1].]

~~2.0~~3.0 Analytical *Testing* Procedure Validation and Analysis Requirements

~~Prior to~~For the implementation of ~~the Analytical *Testing* Procedure~~ATP for the ~~quantification~~analysis of the blood ~~endogenous steroids~~steroid *Markers* in routine *Doping Control* analysis, the Laboratory shall fulfil the following requisites:

- ~~Validate the Analytical *Testing* Procedure, including the determination of the assays' Limit of Quantification (LOQ), Repeatability (s_r), Intermediate Precision (s_w), Bias and Measurement Uncertainty (u_c);~~

a) Validate the Quantitative Procedures (for ITP and CP) for measuring the *Marker* concentrations, as well as the Qualitative Procedure (for CP) for *Marker* identification, as per the ISL *TD* VAL [6] requirements.

~~a)~~b) The ~~Analytical *Testing* Procedure~~validated ATPs shall meet the acceptance values for the parameters of assay performance applicable to the separate ~~determination~~quantification of T and A4 concentrations as specified in ~~Table~~ Article 3.1 ~~below~~.

~~4.1. Analytical *Testing* Procedure Accreditation Requirements~~

- ~~Demonstrate readiness for assay implementation through method validation data and successful participation in at least one *WADA* approved educational External Quality Assessment Scheme (EQAS) round or inter-Laboratory collaborative study. In cases of identified deficiencies, proper corrective action(s) shall be documented and implemented;~~
- ~~Obtain ISO/IEC 17025 accreditation for the Analytical *Testing* Procedure for quantification of endogenous steroids in blood from an Accreditation Body that is a full member of the International Laboratory Accreditation Cooperation (ILAC) and a signatory to the ILAC Mutual Recognition Agreement (ILAC MRA).~~

~~5.0 Analytical *Testing* Procedure and Reporting of Test Results~~

~~5.1. Initial Quantification of the *Markers*~~

- ~~One (1) Aliquot taken from the original "A" *Sample* shall be analyzed once (x1) to quantify T and A4;~~
- ~~QC Sample(s), at low- and high-levels of the *Markers* (see Table 1), shall be included in each initial quantification analytical batch;~~

c) ~~The T and A4 *Marker*~~The Laboratory shall apply the validated ATPs in accordance with the ATP Analysis Requirements specified in Article 3.2.

3.1 ATP Validation Requirements (see also ISL *TD* VAL [6])		
Steroid *Marker*	**Testosterone (T)**	**Androstenedione** (Androst-4-ene-3,17-dione, **A4**),
Identification of *Markers*	The Laboratory shall validate the Qualitative Procedure for confirmation of the identity of the *Markers* in accordance with the requirements of the ISL *TD* IDCR [3] and ISL *TD* VAL [6].	
Limit of Quantification (LOQ)	≤ 0.1 ng/mL	
Working Range	0.1 – 10 ng/mL	
Relative Standard Combined Measurement Uncertainty, u_c (%)	≤ 30% at LOQ ≤ 20% at > 0.3 ng/mL	

3.2 ATP Analysis Requirements		
Steroid *Marker*	**Testosterone (T)**	**Androstenedione** (Androst-4-ene-3,17-dione, **A4**),
Target Steroid *Markers*	Total (free and protein bound) unconjugated fraction	Total (free and protein bound) unconjugated fraction
Test Method and Instrumentation	Quantitative Procedure (ITP and CP): LC-MS^n Qualitative Procedure (CP): LC-MS^n (in compliance with the ISL *TD* IDCR [3])	
Aliquot	The measurement of the T and A4 concentrations shall be conducted (ITP and CP) in singlicate (1x) on one (1) serum Aliquot not greater than (≤) 100 µL.	
Internal Standards	Adequate isotopically labelled internal standards shall be used for both blood steroid *Markers*, *e.g.*, T-d3 (16,16,17-d3) and A4-d3 (19-d3).	
Calibration	Calibration standard(s) shall be included in each sequence of analysis.	
Quality Controls	The QCs shall be prepared either from authentic serum or by spiking standard steroid solution(s), independent from that used for the calibrator(s), into serum. At least one (1) serum QC sample representative of the low part of the working range (*e.g.*, within the first quartile of the working range) and one (1) serum QC sample representative of the high part of the working range (*e.g.*, within the fourth quartile of the working range) shall be used. For the CP, at least one QC sample, depending on the ITP quantification results for the *Markers*, shall be included in each confirmatory analytical batch.	

4.0 Confirmation Procedure for the Blood Steroid *Markers*

4.1 Confirmation Procedure Requests for Blood Steroid *Markers* Triggered through *ADAMS*

a) Once the ITP data of the blood steroid *Markers* is entered and matched with the corresponding *Doping Control* Form (DCF) *in ADAMS*, the Adaptive Model automatically updates the steroidal Passport. If an outlier is identified based on an abnormally high T and/or T/A4 value, an *ATPF*-CPR is triggered and sent automatically to the Laboratory through *ADAMS*. The Laboratory shall ensure the reception and management of CPR notifications using a dedicated *ADAMS* account(s).

b) The TA[iii] shall inform the Laboratory whether to proceed or not with the CP of the blood steroid *Markers*, within fourteen (14) days of the receipt of the *ATPF*-CPR notification.

i. Upon receipt of confirmation to proceed with the CP, the Laboratory shall proceed with the CP of the blood steroid *Markers* as soon as possible.

ii. Any justification from the TA or the Passport Custodian (PC)[iii] to not proceed with the CP shall be provided in writing according to Article 8.6 of the *ISL TD* APMU [7]. In such cases, the Laboratory shall update the Lab Results in *ADAMS* for the *Sample* with a comment stating that the TA or the PC[iii], as applicable, requested to not perform the CP, and the reasons given.

iii. In the absence of communication from the TA or the PC[iii] within fourteen (14) days from the *ATPF*-CPR notification, the Laboratory shall proceed with the CP of the blood steroid *Markers*.

c) When the Laboratory receives an *ATPF*-CPR for a *Sample* for which *Adverse Analytical Finding*(s) (*AAF*) have been reported for other *Prohibited Substance*(s) or *Prohibited Method*(s), the Laboratory shall consult the TA about the need to conduct the CP for the blood steroid *Markers*

4.2 Confirmation Procedure Requests from the *Testing* Authority, the Passport Custodian, the *Athlete* Passport Management Unit, or *WADA*.

The Adaptive Model will also flag abnormally low or variable steroid *Markers*. However, in such cases the Laboratory will not receive an automatic notification through *ADAMS*. Instead, the APMU will advise the PC (who will advise the TA, if different) on whether the *Sample*, or other *Samples* from the corresponding Passport, shall be subjected to a CP for the blood steroid *Markers*. Therefore, in these cases the Laboratory shall receive a written request from the TA[iii], or *WADA*, before proceeding with the CP.

4.3 Confirmation of Blood Steroid *Marker* Values

The CP for the blood steroid *Markers* following receipt of an *ATPF*-CPR, or upon request, consists of the application of the same ATP (see Article 3) for the quantification of the concentrations of T and A4 in the ITP as well as their identification (in compliance with the ISL *TD* IDCR [3]).

5.0 Reporting Initial *Testing* Procedure and Confirmation Procedure Results for the Blood Steroid *Markers*

a) The T and A4 concentrations shall be reported in *ADAMS* in nanograms per milliliter (ng/mL~~);~~).

[iii] The APMU or PC, where the PC is not the TA, may contact, in writing, the Laboratory regarding performance of a CP of the blood steroid *Markers* on behalf of the TA. In such cases, the APMU (which may have been bestowed such authority by the PC) or the PC shall copy the relevant TA.

[Comment: for to Article 5.0 a): For the purposes of the Steroidal Module of the ABP, the T/A4 ratio does not need to be calculated or reported by the Laboratory; it will be automatically calculated in ADAMS].

b) If the measured *Marker* concentration(s) of T and/or A4 is below the LOQ of the assay, the Laboratory shall report a value of "-1" for itsthe affected concentration in *ADAMS*.

b)c) If the concentration(s) of T and/or A4 cannot be determined or the *Marker*(s) cannot be identified when performing the CP, the affected *Marker(s)* shall be reported as "-1" and the Laboratory shall make a corresponding comment in the Test Report on why the *Marker* could not be quantifiedLab Results in *ADAMS* (*e.g.,* the measurement of the *Marker* is not possible due to unusual matrix interferences);).

- An observation of hemolysis of the *Sample* should be recorded in the comments section of the Laboratory Test Report in *ADAMS*.

5.2. Confirmatory Quantification of the *Markers*

If requested by the *Testing* Authority (TA), *Results Management* Authority (RMA) or *WADA*, the Laboratory shall proceed with the confirmatory quantification of the *Markers* of the blood Steroidal Module.

[Comment: An APMU or Passport Custodian (PC), where the PC is not the TA, may request a confirmatory quantification on behalf of the TA or RMA. In such cases, the APMU or PC shall copy the relevant TA or RMA, as applicable, on all written requests to the Laboratory for confirmatory quantification.]

When a confirmatory quantification analysis is requested:

- One (1) new Aliquot taken from the original "A" *Sample* shall be analyzed once (x1) to identify (as per the TD IDCR [8]) and to quantify T and A4.
- At least one QC Sample (see Table 1), depending on initial quantification results, shall be included in each confirmatory quantification analytical batch;
- The T and A4 *Marker* concentrations shall be reported in *ADAMS* in nanograms per milliliter (ng/mL).
- If the measured *Marker* concentration is below the LOQ of the assay, the Laboratory shall report a value of "-1" for its concentration in *ADAMS* and the Laboratory shall make a comment in the Test Report on why the *Marker* could not be quantified (e.g., the measurement of the *Marker* is not possible due to unusual matrix interferences);
- An observation of hemolysis of the *Sample* should be recorded in the comments section of the Laboratory Test Report in *ADAMS*.

Table 1: Analytical *Testing* Procedure Validation and Performance Requirements for the initial and confirmatory quantification of blood (serum) endogenous steroid *Markers*.

Markers	**Testosterone (T)**, total unconjugated fraction **Androstenedione** (Androst-4-ene-3,17-dione, **A4**), total unconjugated fraction
Method and Instrumentation	Liquid Chromatography combined with tandem Mass Spectrometry based on triple quadrupole or HRMS analyzer (LC-MS^n; n ≥ 1).
Range of the Method	Shall cover the ranges of *Marker* concentrations normally found in males and females and demonstrate linearity between **0.1 – 10 ng/mL (~ 0.35 – 35 nmol/L)**, at least.
Limits of Quantification (LOQ)	The LOQ shall be determined during method validation and is defined as the lowest concentration with an associated u_c (%) not greater than (≤) 30% and shall be not greater than (≤) **0.1 ng/mL (~ 0.35 nmol/L)**.
Relative Standard Combined Measurement Uncertainty, u_c (%)	The estimated u_c (%) shall be no greater than (≤) **30%** at the LOQ; and not greater than (≤) **20%** when the *Marker* concentration is greater than (>) 0.3 ng/mL.
Sample	*Marker* quantification shall be conducted on one serum Aliquot of no greater than (≤) **100 µL**.
Internal Standards	Adequate isotopic-labelled internal standards shall be used for both *Markers* (*e.g.*, Testosterone-d3 (16,16,17-d3)[iv] and Androstenedione-d3 (19-d3)[v]).
Calibration	Calibration standard(s) shall be included in each sequence of analysis. The "*Multilevel Serum Calibrator Set*" from Chromsystem[vi] is recommended. Other calibrators may be used as long as the method performance criteria are met.
Quality Control	At least two (2) quality control (QC) samples in serum containing representative low (*e.g.*, 0.5 ng/mL) and high (*e.g.*, 5 ng/mL) concentrations of the *Markers* shall be included in each analytical batch. The QCs should be prepared from authentic samples, or by spiking with a standard solution independent from that used for the calibrator(s).

[A]pplicable links:
[iv] https://www.lipomed-usa.com/en/testosterone-d3, for example.
[v] https://www.lgcstandards.com/US/en/Androstenedione-d3/p/TRC-A637552-1MG, for example.
[vi] https://chromsystems.com/en/6plus1r-multilevel-serum-calibrator-set-masschromr-steroid-panel-2-72039.html

~~6.0 Bibliography~~

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~~2. Elings Knutsson J, Andersson A, Baekken LV, Pohanka A, Ekström L, Hirschberg AL. Disposition of Urinary and Serum Steroid Metabolites in Response to Testosterone Administration in Healthy Women. *J Clin Endocrinol Metab*. 2021;106(3):697-707. doi:10.1210/clinem/dgaa904~~

~~3. Salamin O, Nicoli R, Langer T, et al. Longitudinal evaluation of multiple biomarkers for the detection of testosterone gel administration in women with normal menstrual cycle. *Drug Test Anal*. Published online April 5, 2021. doi:10.1002/dta.3040~~

~~4. Ponzetto F, Mehl F, Boccard J, et al. Longitudinal monitoring of endogenous steroids in human serum by UHPLC-MS/MS as a tool to detect testosterone abuse in sports. *Anal Bioanal Chem*. 2016;408(3):705-719. doi:10.1007/s00216-015-9185-1~~

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~~6. Handelsman DJ, Bermon S. Detection of testosterone doping in female athletes. *Drug Test Anal*. Published online September 3, 2019:dta.2689. doi:10.1002/dta.2689~~

~~7. World Anti-Doping Agency. International Standard for Testing and Investigations. Published 2023. Accessed June 6, 2023. https://www.wada-ama.org/en/resources/world-anti-doping-program/international-standard-testing-and-investigations-isti~~

~~8. World Anti-Doping Agency. *Technical Document - Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for Doping Control Purposes*.; 2023. Accessed January 24, 2023. https://www.wada-ama.org/en/resources/lab-documents/td2023idcr#resource-download~~

6.0 References

[1] The World Anti-Doping *Code International Standard* for Laboratories.

[2] *WADA Technical Document* ISL *TD* ATP: Analytical *Testing* Procedures

[3] *WADA Technical Document* ISL *TD* IDCR: Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the *Identity* of Analytes for *Doping Control* Purposes.

[4] The World Anti-Doping *Code International Standard* for *Testing.*

[5] *WADA Technical Document* ISL *TD* LCOC: Laboratory Chain of Custody

[6] *WADA Technical Document* ISL *TD* VAL: Minimum Requirements for Validation of Analytical *Testing* Procedures for *Doping Control.*

[7] *WADA Technical Document* ISL *TD* APMU: Athlete Passport Management Unit Requirements and Procedures

[Comment to Article 6.0: Current versions of WADA International Standards and Technical Documents may be found at https://www.wada-ama.org/en/what-we-do/international-standards]