WADA Technical Document – ~~TD2021EAAS~~ISL TD2027USM

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Written by: Reviewed by:	*WADA* Science/EAAS Working Group *WADA* Steroidal ABP WG/ Laboratory Expert Advisory Group	Approved by:	*WADA* Executive Committee
Date:	~~20 May 2021~~17 March 2026	Effective ~~Date~~date:	1 ~~June 2021~~January 2027

~~Measurement~~Analytical and Reporting ~~of Endogenous Anabolic Androgenic Steroid (EAAS)~~ Requirements for the Urinary *Markers* of the ~~Urinary Steroid Profile~~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 ~~measurement~~analysis and reporting of the ~~"steroid profile" of urine *Samples* in support~~urinary *Markers* of the ~~steroidal module~~Steroidal Module of the *Athlete Biological Passport* (*ABP*) ~~(~~to uncover the ~~steroidal Passport).~~

~~1.1 The Steroid Profile~~

~~The measurement~~*Use* of ~~steroidal *Markers* [concentrations and ratios~~synthetic forms of ~~defined~~ Endogenous Anabolic Androgenic Steroids (EAAS~~)] in a urine *Sample* form the steroid profile for that *Sample* (see Table 1).~~

~~The steroid profiles of a series of urine *Samples* collected from an *Athlete* over a period of time constitute the steroidal Passport of that *Athlete*.~~

> ~~**The administration of synthetic forms of EAAS can alter one or more of the *Markers* of the urinary steroid profile, resulting in increased or decreased concentrations and/or ratios of specific pairs of steroid *Markers*[1-3]. This effect forms the basis for the use of the steroidal Passport as a tool for the detection of doping with EAAS,**~~), in particular testosterone ~~**(T),**~~and its precursors ~~**(for example, 4-androstenediol, androstenedione and prasterone), its active *Metabolite* [dihydrotestosterone (DHT)], or its epimer epitestosterone (E).**~~

~~The steroidal module of the *ABP* utilizes the Adaptive Model in *ADAMS* to trigger *Atypical Passport Findings* (*ATPFs*), which can lead to the performance of Confirmation Procedures (CP), *Target Testing* of an *Athlete*, or to establish *Use* of a *Prohibited Substance* and/or *Prohibited Method* as per *Code* Article 2.2 (see *International Standard* for *Results Management*, Annex C[4]).~~

~~1.2~~1.1 Procedure for ~~Determination~~Analysis of the Urinary Steroid ~~Profile~~*Markers*

~~Each urine *Sample* shall be analyzed to determine its steroid profile. The determination and reporting of a *Sample*'s steroid profile follows a two-step procedure:~~

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-The Analytical *Testing* Procedure (ATP) applied to the analysis of the urinary steroid *Markers* involves the measurement of the urinary concentrations of six (6) naturally occurring EAAS, namely Testosterone (T), its *Metabolites* [Androsterone (A), Etiocholanolone (Etio), 5α-Androstane-3α,17β-diol (5αAdiol) and 5β-Androstane-3α,17β-diol (5βAdiol)], and its epimer epitestosterone (E). The ratios of the urinary steroid *Markers* concentrations (see Table 1) are automatically calculated in ADAMS, with the exception of the T/E ratio, which is reported directly by the Laboratory.

a) The ATP for the urinary steroid *Markers* is a mandatory ATP (see ISL *TD* ATP [2]) and, therefore, it is applied to all urine *Samples*.

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

~~i.~~ An Initial *Testing* Procedure (ITP) ~~is conducted to estimate~~based on the ~~steroid profile of the *Sample*, and~~

i. ~~A subsequent CP is performed when~~ Gas Chromatography-Mass Spectrometric (GC-MS^n) quantification of the urinary steroid *Markers* (see Article 2.1). Substances which may impact the ~~reported steroid profile constitutes an *ATPF*, as determined by the Adaptive Model, or upon request from~~urinary steroid *Markers* (see Article 2.1.2) shall also be included in the relevant ITP to support steroidal Passport interpretation by the *Athlete* Passport Management Unit (APMU), ~~the *Testing* Authority or *WADA*.~~ and

ii. A subsequent Confirmation Procedure (CP) (see Article 2.2), which consists of the GC-MS^n quantification and identification (as per ISL *TD* IDCR [3]) of the urinary steroid *Markers* and the eventual performance of Gas Chromatography/Combustion/Isotope Ratio Mass Spectrometry (GC/C/IRMS) analysis (see ISL *TD* IRMS [4]). The CP is performed when an elevated T/E ratio in the *Sample* constitutes an outlier in the corresponding Passport, 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 2.2.1 b).

[Comment to Article 1.1: 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].]

Table 1. Urinary *Markers* of the ~~Urinary Steroid Profile~~Steroidal Module of the *ABP*.

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~~Type of *Marker*~~	Urinary Steroid ~~Profile~~ *Markers*	Determination
~~Concentrations of Steroids~~EAAS concentrations	- Testosterone (T) - Epitestosterone (E) - Androsterone (A~~);~~) - Etiocholanolone (Etio~~);~~) - 5~~α-~~α-Androstane-3~~α,~~α,17β-diol (5~~αα~~Adiol~~);~~), and - ~~5~~β-Androstane-3~~α,~~α,17β-diol (5βAdiol~~);~~ - ~~Testosterone (T); and~~ - ~~Epitestosterone (E).~~)	Determined by the Laboratory by GC-MS^n from the combination of the free ~~steroid fraction and the conjugated fraction~~ and glucuronidated steroids (released after hydrolysis with an enzyme with substrate specificity for β-D-glucuronide linkages, such as purified β-glucuronidase from *E. coli*, or other Fit-for-Purpose enzymes with the same substrate specificity, as determined by the Laboratory during Test Method validation).
Ratios of ~~Steroids~~EAAS concentrations	- T/E ratio	~~As reported~~Determined by the Laboratory ~~in *ADAMS*~~based on T and E concentrations.
	- ~~A/T;~~ - 5αAdiol/E - A/Etio~~;~~ - ~~5~~ααAdiol/5βAdiol~~; and~~ - ~~5αAdiol/E~~	Automatically computed in *ADAMS* from respective ~~steroid~~measured and reported EAAS concentrations ~~after the reporting of the steroid profile by the Laboratory~~.

~~1.3 Factors Impacting the Steroid Profile~~

~~In addition to the effects mediated by the administration of EAAS, alteration of the urinary steroid profile can occur for a number of other reasons including, but not limited to, the following factors [1-3]:~~

- ~~Intake of alcohol (ethanol);~~
- ~~The administration of other anabolic androgenic steroids (*e.g.* stanozolol);~~
- ~~The administration of human chorionic gonadotrophin (hCG) in males;~~
- ~~The administration of aromatase inhibitors and anti-estrogenic substances;~~

- ~~The administration of inhibitors of 5α reductase (*e.g.* finasteride, dutasteride);~~
- ~~The administration of ketoconazole or other similar compounds (*e.g.* fluconazole, miconazole);~~
- ~~The use of masking agents (*e.g.* probenecid) and diuretics;~~
- ~~Microbial activity;~~
- ~~*Sample* manipulation.~~

2.0 Analytical *Testing* Procedure Requirements

~~1.4~~2.1 Initial *Testing* Procedure ~~(ITP)~~Requirements

~~1.5~~ The ITP ~~Method Requirements~~

~~The quantification of~~analysis for the urinary steroid *Markers* ~~of the steroid profile~~ shall be based on ~~gas chromatography combined with mass spectrometry (a~~ GC-MS^n~~; *n* ≥ 1).~~

~~**Table 2**. Requirements of the ITP for Quantification of the *Markers* of the Steroid Profile~~ Quantitative Procedure.

2.1.1 ~~2.1.1~~ ITP Validation Requirements (see also ISL *TD* VAL [5])	
Working Range ~~of the Method~~	~~Shall cover~~ The working range of the ~~ranges of *Marker*~~Quantitative Procedure shall be investigated at the concentrations of the urinary steroid *Markers* normally found in ~~males and females~~*Athletes' Samples*.
~~**Enzymatic Hydrolysis**~~	~~Assess the efficiency of the enzymatic hydrolysis using β-glucuronidase from *E. coli*~~
~~**Derivatization**~~	~~Assess the efficiency of the trimethylsilyl (TMS) derivatization~~
~~**Limits**~~**Limit of Quantification (LOQ)**	The LOQ shall be determined during ~~method~~Test Method validation as the lowest concentration that can be measured with an u_c (%) not greater than (≤) 30% and shall meet the following criteria: • T, E ≤ 1 ng/mL~~;~~

	• 5~~α~~α-Adiol, 5~~β~~β-Adiol ≤ 10 ng/mL~~;~~ • A, Etio ≤ 500 ng/mL						
Maximum allowed Relative Combined Standard Measurement Uncertainty, u_{c_Max} (%)	**Level**	**A**	**Etio**	**T**	**E**	**Adiols (5~~α~~α-, 5~~β~~β-)**	**T/E**
		~~The estimated $u_{c\_}$(%) shall be not greater than (≤) the u_{c_Max} (%) value given below~~					
	at LOQ	≤ 30%					
	at ≥ 5 x LOQ	≤ 20%				≤ 25%	
	~~(~~[T_T] and [E_T] > 5 ng/mL						≤ 15%
	~~(~~[T_T] and/or [E_T] ≤ 5 ng/mL						≤ 30%
2.1.2 ~~2.1.2~~ ITP Analysis Requirements							
***~~Sample~~*Quantitative Procedure**	a) The ~~ITP for the quantification~~concentrations of the ~~*Markers* of the~~urinary steroid ~~profile~~*Markers* shall be ~~conducted on a single~~measured in one (1) Aliquot~~.~~ by a GC-MS^n Quantitative Procedure. Appropriate isotopically-labelled preparations of urinary steroid *Marker*(s) shall be used as internal standards. b) When needed, the volume of the Aliquot may be adjusted, for example, as a function of its ~~specific gravity~~Specific Gravity (SG~~) and~~). c) In each sequence of analysis, calibration standard(s) shall be included. ~~a)~~d) At least one (1) urine QC sample representative of the low part of the working range (*e.g.*, within the ~~sex~~first quartile of the ~~*Athlete*~~working range) and one (1) urine QC sample representative of the high part of the working range (*e.g.*, within the fourth quartile of the working range) shall be used.						
~~Calibration~~	~~Calibration standard(s) or a calibration curve shall be included in each sequence of analysis.~~						
~~Quality Control~~	~~At least two (2) quality control (QC) urine samples containing representative low and high concentrations of the *Markers* of the steroid profile shall be included in each sequence of analysis.~~						

Enzymatic Hydrolysis	~~Purified~~An enzyme with substrate specificity for β-D-glucuronide conjugates and minimizing the risk of conversion of endogenous steroids *(e.g.,* purified β-glucuronidase from *E. coli*) shall be used for the hydrolysis of the ~~glucuroconjugated~~glucuronide-conjugated urinary steroids~~, and the completeness~~. The efficiency of hydrolysis shall be ~~monitored~~controlled in each Aliquot ~~with isotopically labeled A-glucuronide (or an equivalent scientifically recognized alternative). *H. pomatia* mixtures shall not be used~~.
Derivatization	The ~~*Markers* of~~urinary steroid ~~profile~~*Markers* shall be ~~analyzed~~determined as TMS derivatives (TMS enol ethers and/or TMS ethers). ~~Completeness~~The efficiency of the derivatization shall be controlled in each Aliquot through the monitoring of mono-O-TMS vs. di-O-TMS derivative of A.
~~**T/E Ratio**~~	~~The T/E ratios shall be determined from the ratios of chromatographic peak areas or peak heights after correction against a calibrator or a calibration curve.~~
Factors ~~Impacting~~impacting the ~~Steroid Profile~~urinary steroid *Markers*	The Laboratory shall: a) Monitor for signs of microbial activity [*e.g.* presence of indicators of 3~~α~~-α-hydroxysteroid dehydrogenase (HSD) activity~~];~~]. *[Comment: The effect of microbial contamination may increase when direct enzymatic hydrolysis ~~of~~is applied to urine Samples ~~may increase the effects of microbial contamination~~.]* b) Test for the presence of ~~conjugated~~the following non-prohibited substances: i. Conjugated *Metabolite*(s) of ethanol [*e.g.*~~.~~., ethanol glucuronide (EtG)], and ~~i.~~ii. 5~~α~~-α-reductase inhibitors (*e.g.*~~—~~., *Metabolites* of finasteride~~,~~ and dutasteride~~) and ketoconazole (and similar substances~~).

2.1.3 Reporting Initial *Testing* Procedure Results for the ~~*Sample*'s~~Urinary Steroid ~~Profile from the ITP~~*Markers*

Following the performance of the ITP, the Laboratory shall report in *ADAMS* the measured concentrations of the urinary steroid ~~profile~~*Markers* and the T/E ratio for each *Sample* analyzed.

The Laboratory shall report ~~in *ADAMS*~~:

a) The SG of the *Sample,* as determined by the Laboratory (see ISL *TD DL* [5]~~);~~[6]).

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b) The uncorrected concentrations of T, E, A, Etio, 5~~α~~αAdiol and 5βAdiol, ~~and the T/E ratio;~~expressed in nanograms per milliliter (ng/mL).

[Comment~~:~~ to Article 2.1.3 b): When the ITP ~~measurement~~analysis of a urinary steroid ~~profile~~ Marker is not possible due to, for example, dilution, unusual matrix interferences, signs of microbial contamination, inhibition of ~~the~~ enzymatic hydrolysis or incomplete derivatization, the Laboratory ~~should~~shall repeat the analysis with an alternative Sample preparation procedure (e.g.~~:~~., changing Aliquot volumes, application of ~~solid phase extraction,~~Solid Phase Extraction (SPE), or extraction with a different solvent).

~~If, however, a Marker of the steroid profile cannot be quantified,~~If the concentration ~~of~~cannot be determined, the affected urinary steroid Marker shall be reported as "-1". The Laboratory shall make a corresponding comment in the ~~Test Report on why this Marker could not be quantified~~Lab Results in ADAMS (e.g.~~:~~., < LOQ, incomplete derivatization).

~~*When the chromatographic peak signal for a Marker cannot be detected (i.e. is below the detection capability of the assay), the concentration of the Marker shall be reported as "-2" (See Table 3 for reporting of specific situations for [T], [E], and T/E).*~~

The Laboratory may also provide information on other ~~steroidal parameters~~steroids such as ~~prasterone (DHEA), dihydrotestosterone (~~DHEA and DHT~~) and 6α-hydroxy-androstenedione (6α-OH-AD)~~ at the request of the Testing Authority, ~~Results Management Authority or~~ (TA), the Passport Custodian (PC), the APMU or WADA.]

c) ~~Any signs~~The T/E ratio (calculated from measured T and E concentrations).

~~c)~~d) The concentrations of the indicators of microbial activity ~~in the *Sample*, e.g. ratios of 5α-~~5α-androstanedione (5~~α~~αAND) ~~to A~~ and 5β-androstanedione (5βAND~~) to~~), expressed in ng/mL. The ratios 5aAND/A and 5βAND/Etio~~, as determined from the respective steroid concentrations;~~ will be automatically calculated in *ADAMS*.

~~d)~~e) The presence ~~or absence~~ in the *Sample* of non-prohibited substance(s) that may alter the urinary steroid ~~profile (see Article 1.3).~~*Markers*. The Laboratory shall report the estimated ~~levels of~~concentrations of the following substances:

i. EtG ~~if~~ ≥ 5 ~~μ~~μg/mL~~;~~ (in micrograms per milliliter, μg/mL).

~~• Carboxy-finasteride if ≥ 5 ng/mL;~~

ii. , 4-hydroxy- and/or 6-hydroxy-dutasteride ~~if ≥ 5~~(in ng/mL~~;~~).

~~• Ketoconazole if ≥ 100 ng/mL;~~

~~• Fluconazole if ≥ 500 ng/mL;~~

~~• Miconazole if ≥ 1,000 ng/mL.~~

~~2.2.1~~*[Comment to Article 2.1.3 e) ii.: For harmonization purposes, an MRPL at 5 ng/mL is established for these 5α-reductase inhibitors; however, the MRPL is not a reporting limit and, therefore, the Laboratory may report these compounds at concentrations below the MRPL if detected in a Sample.]*

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2.1.4 *Sample* Validity for the Steroidal Module of the ~~*Sample* Steroid Profile~~*ABP*

The validity of the urine *Sample* for the Steroidal Module of the *ABP* will be determined automatically upon reporting the measured concentrations of the urinary steroid ~~profile~~*Markers* in *ADAMS*. A urine *Sample* will be invalid for the *ABP* only when ~~the *Sample* shows~~ signs of extensive degradation are observed, as determined by:

a) 5~~α~~(α)AND/A ≥ 0.1, and/or

b) 5βAND/Etio ≥ 0.1

[Comment to Article 2.1.4: In addition, following the reporting of the urinary steroid ~~profile~~Markers in ADAMS by the Laboratory, the validity of the Sample may be ~~evaluated as "invalid"~~modified by the APMU upon review of the ~~steroid profile~~ data~~, for~~. For example, the APMU could invalidate a Sample for the ABP by considering the presence of substances that may alter the concentrations of the urinary steroid ~~profile~~Markers in the Sample~~.~~] (see ISL TD APMU [7]).]

Table 32. Summary of conditions for reporting [T] and [E ~~concentrations and T/E ratio.~~]

~~*Marker(s)*~~	~~**Concentration of T**~~**Reported [T]**	~~**Concentration of E**~~**Reported [E]**	**Reported T/E ~~ratio~~**
[T] and [E] higher than or equal to (≥) $LOQ_{(T)}$ and $LOQ_{(E)}$, respectively	~~Chromatographic peak signal of T measured at or above (≥) the LOQ.~~ ~~[T] ≥ $LOQ_{(T)}$~~ Report [T] as measured	~~Chromatographic peak signal of E measured at or above (≥) LOQ.~~ ~~[E] ≥ $LOQ_{(E)}$~~ Report [E] as measured~~.~~	Report T/E ~~(as determined by the Laboratory from corrected peak heights/areas)~~based on measured [T] and [E]
	~~Chromatographic peak signal of E detected, but below (<) LOQ.~~ ~~$LOD_{(E)}$ ≤ [E] < $LOQ_{(E)}$~~ ~~**Report E as "-1"**~~		
	~~Chromatographic peak signal of E not detected.~~ ~~[E] < $LOD_{(E)}$~~ ~~**Report E as "-2"**~~	~~**Report T/E as "-1"**~~ ~~Report the $LOD_{(E)}$~~ ~~*Comment in ADAMS:*~~ ~~*T/E ratio could not be measured accurately because E could not be detected.*~~	

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~~Chromatographic peak signal of [T detected, but below (<)]~~ and/or [E] not measured in the *Sample*

[T] lower than (<) LOQ.

$LOD_{(T)} \leq [T] < LOQ_{(T)}$

- **~~Report T as "-1"~~**

-1

~~Chromatographic peak signal of E measured at or above (≥) LOQ.~~

$[E] \geq LOQ_{(E)}$

Report [E] as measured

~~Report T/E~~

~~(as determined by the Laboratory from corrected peak heights/areas)~~

-1

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	~~Chromatographic peak signal of E detected, but below (<) LOQ.~~ $LOD_{(E)} \leq [E] < LOQ_{(E)}$ **~~Report E as "-1"~~**	
	~~Chromatographic peak signal of E not detected.~~ $[E] < LOD_{(E)}$ **~~Report E as "-2"~~**	**~~Report T/E as "-1"~~** *~~Comment in ADAMS:~~* *~~T/E ratio could not be measured accurately because the concentration of T could not be measured, and E could not be detected~~*

~~Chromatographic peak signal of T not detected.~~

$[T] < LOD_{(T)}$

- **~~Report T as "-2"~~** [E] lower than (<) $LOQ_{(E)}$

~~Chromatographic peak signal of E measured at or above (≥) LOQ.~~

$[E] \geq LOQ_{(E)}$

Report ~~E~~[T] as measured

-1

Report T/E ~~as "-1"~~ ~~Report the $LOD_{(T)}$~~

~~Comment in ADAMS:~~

*~~T/E ratio could not be~~*based on measured *~~accurately because T could not be detected~~* [T] and LOQ of E (T/LOQ_E)

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~~Chromatographic peak signal of [T] and [E detected but below] lower than~~ (<) LOQ.

$LOD_{(E)} \leq [E] <_{(T)}$ and $LOQ_{(E)}$

- **~~Report E as "-1"~~**, respectively

~~Report T/E as "-1"~~

~~Report the $LOD_{(T)}$~~

~~Comment in ADAMS:~~

~~T/E ratio could not be measured because T could not be detected, and E could not be measured.~~ -1

-1

-1

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	~~Chromatographic peak signal of E not detected.~~	**~~Report T/E as "-2"~~** ~~Report the $LOD_{(E)}$ and $LOD_{(T)}$~~

	~~[E] < LOD(E)~~ **~~Report E as "-2"~~**	~~*Comment in ADAMS:*~~ ~~*T/E ratio could not be measured because T and E could not be detected.*~~

~~1.6~~2.2 Confirmation ~~Procedures (CP)~~Procedure Requirements

~~The CP for the EAAS *Markers* include the GC-MS^n ($n \geq 1$) identification (in compliance with the TD IDCR [6]) and quantification, as well as the GC/*C*/IRMS analysis [7] of the *Marker(s)* of the steroid profile.~~

~~In addition, the Laboratory shall confirm the presence or absence of factors impacting the steroid profile (see Article 1.3).~~

2.2.1 ~~CP~~Confirmation Procedure Requests ~~(CPRs)~~

a) ~~3.1.1 CPRs~~Confirmation Procedure Requests triggered by *Atypical Passport Findings* ~~(*ATPF*)~~ through *ADAMS*

i. Once the ~~*Sample*'s steroid profile~~ITP data ~~are~~of the urinary steroid *Markers* is entered ~~in *ADAMS*~~ and matched with ~~an *Athlete*~~the corresponding *Doping Control* Form (DCF) *in ADAMS*, the Adaptive Model automatically updates the steroidal Passport. If an *ATPF* is identified based on an abnormally high T/E value, ~~a CP request (~~an *ATPF*-CPR~~)~~ is triggered and sent automatically to ~~Laboratories~~the Laboratory through *ADAMS*. The Laboratory shall ensure the reception and management of *ATPF*-CPR notifications using a dedicated *ADAMS* account(s).

ii. ~~Upon receipt of an *ATPF* CPR,~~The TA[1] shall inform the Laboratory ~~shall~~whether to proceed or not with the CP of the urinary steroid ~~profile~~*Markers*, as soon as possible~~, unless the presence of ethanol or other factors impacting the steroid profile has been detected in the *Sample*. In such cases, the Laboratory shall receive, within fifteen (15~~ and no later than fourteen (14) days from the receipt of the *ATPF*-CPR notification~~, an advice from the Passport Custodian or the *Testing*~~

[1] The APMU or PC, where the PC is not the TA, may contact, in writing, the Laboratory regarding performance of a CP of the urinary 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.

~~Authority (or *Results Management* Authority, if different) on whether to proceed or not with the CP of the *Sample*'s steroid profile~~.

- ~~*[Comment: In the absence of communication from the Passport Custodian or the Testing Authority (or Results Management Authority) within fifteen (15) days from the ATPF-CPR notification*~~Upon receipt of the confirmation to proceed with the CP, the Laboratory shall proceed with the CP of the urinary steroid ~~*profile (see Article 3.2)].*~~*Markers* as soon as possible.

Any justification from the ~~Passport Custodian~~TA or the ~~*Testing* Authority (or *Results Management* Authority)~~ PC[1] to not ~~to~~ proceed with the CP shall be provided in writing ~~and in compliance with the~~according to Article 8.6 of the ISL *TD* APMU [~~8~~].

- ~~*[Comment:*~~[7]. In such cases ~~*when the Laboratory is instructed by the Passport Custodian or the Testing Authority (or Results Management Authority) not to perform the CP*~~, the Laboratory shall update the Lab Results in *ADAMS* ~~*Test Report*~~ for the *Sample* with a comment stating that the ~~*Passport Custodian, Testing Authority (or Results Management Authority)*~~TA or the PC[1], as applicable, requested to not ~~to~~ perform the CP, and the reasons given~~.]~~.
- In the absence of communication from the TA or the PC[1] within fourteen (14) days from the *ATPF*-CPR notification, the Laboratory shall proceed with the CP of the urinary steroid *Markers*.

iii. 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 ~~*Testing* Authority~~TA (or ~~*Results Management* Authority~~RMA, if different) about the need to conduct the CP for the ~~*Markers* of the~~urinary steroid ~~profile~~*Markers*.

b) ~~3.1.2 CPRs~~Confirmation Procedure Requests from the ~~APMU, the~~ *Testing* Authority ~~(or *Results*~~, the Passport Custodian, the *Athlete* Passport Management ~~Authority, as applicable)~~Unit or *WADA*.

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The Adaptive Model will also ~~determine~~identify abnormal values ~~or sequences~~ of the other ~~ratios of the "~~urinary steroid ~~profile" (A/T~~*Markers* ratios (5αAdiol/E, A/Etio~~,~~ and 5α~~α~~Adiol/5βAdiol~~, 5αAdiol/E~~). However, in such cases the Laboratory will not receive an automatic ~~"~~*ATPF*-CPR~~"~~ notification through *ADAMS*. Instead, the APMU will advise the ~~*Testing* Authority (or *Results Management* Authority~~PC (who will advise the TA, if different) on whether the *Sample* shall be subjected to ~~CP. Therefore, in these~~ a CP for the urinary steroid *Markers*. In such cases, the Laboratory shall receive a written request from the ~~*Testing* Authority (or *Results Management* Authority, if different)~~TA[1], or *WADA*, before proceeding with the CP.

~~In the absence of an *ATPF* CPR, requests for CP can be made also by the *Testing* Authority (or *Results Management* Authority, if different), the APMU *, or *WADA*.~~

~~* where the respective client of the APMU has agreed to bestow such authority to the APMU.~~

~~3.1 CP Test Methods~~

~~3.2.1 CP of Steroid Profile *Markers* by GC-MS^n~~

2.2.2 Confirmation Procedure

a) The CP for the urinary steroid *Markers* includes the GC-MS^n quantification and identification (in compliance with the ISL *TD* IDCR [3]), as well as the possible GC/C/IRMS analysis (see ISL *TD* IRMS [4]), of the urinary steroid *Markers*.

In addition, the CP shall include the estimation of the EtG concentration. Upon request from the TA[1] or *WADA*, the presence of 5α-reductase inhibitors shall also be confirmed.

The Laboratory shall ~~quantify all the *Markers* of the steroid profile in one Aliquot by a validated Fit-for-Purpose GC-MS^n ($n \geq 1$) quantification method. Identification (in compliance with the TD IDCR [6]) of the *Markers* that triggered the CP shall be performed as well.~~

~~a)~~b) ~~In every case, the Laboratory shall~~ confirm quantitatively all the ~~*Markers* of the~~urinary steroid ~~profile~~*Markers* before proceeding with the GC/C/IRMS analysis~~;~~, except if:

i. ~~*[Comment: This requirement does not apply if the Testing Authority (or Results Management Authority, as applicable)*~~The TA has authorized the Laboratory to proceed with the confirmation analysis of the urinary steroid *Markers* and the GC/C/IRMS regardless of the ITP results, or

~~i.~~ii. The TA has exceptionally authorized the Laboratory to proceed directly to GC/C/IRMS analysis without ~~*a need for a*~~the quantitative confirmation of the urinary steroid *Markers* (for example, in cases of limited *Sample* volume).

~~*For T/E values, only T needs to be confirmed if E is not detected or the volume of the Sample is not sufficient.]*~~

~~In the case of~~

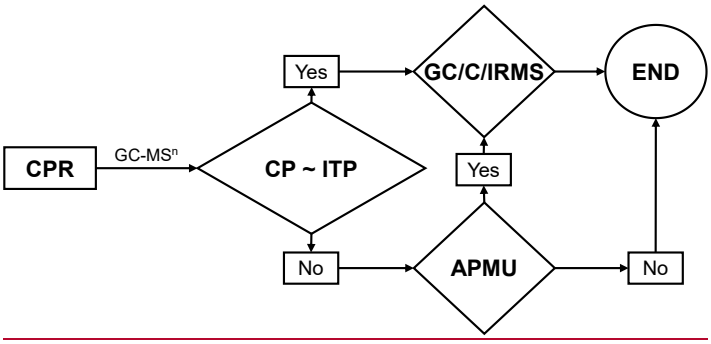

Figure 1: Steps for the GC-MS^n Confirmation Procedure and GC/C/IRMS analysis of the urinary steroid *Markers*.

However, GC/C/IRMS analysis is not mandatory when, following an *ATPF*-CPR for an abnormally high T/E ratio, ~~GC/C/IRMS analysis is not mandatory when~~ the confirmed T/E value is below the ~~confirmation T/E cut off~~upper tolerance limit calculated by the Adaptive Model ~~and~~ provided within the *ATPF*-CPR notification received from *ADAMS*~~;~~.

~~• For other CP requests, when the steroid profile CP does not confirm the ITP values that triggered the CP (e.g. 5αAdiol/E value), taking into consideration the expanded uncertainty of the measurement ($U_{95\%}$, $k = 2$), the Laboratory shall consult the *Testing* Authority to determine if the GC/C/IRMS analysis is necessary. In~~

~~the event that GC/C/IRMS analysis is deemed unnecessary, the Laboratory shall update the *ADAMS* report for the *Sample* with the confirmed values of all the *Markers* of the steroid profile and include a comment that GC/C/IRMS analysis was not necessary.~~

~~*[Comment: for ratios other than the T/E, the u_c (%) of the ratio shall be calculated by propagation of uncertainties of the corresponding Marker concentrations.]*~~

~~b)~~c) ~~The same~~ The validation and analytical requirements presented in ~~Table~~Article 2.1 for the GC-MS^n ITP shall also apply for the GC-MS^n CP, with the ~~following~~ modifications~~:~~ described in Articles 2.2.2.1 and 2.2.2.2 below.

~~• GC-MS^n CP Validation Requirements~~

~~- For determinations of A, Etio, 5α-Adiol and 5β-Adiol, the u_c (%) shall be not greater than (≤) 15% when the concentrations are five times (5x) the respective LOQ;~~

~~- For determinations of T, E and T/E ratios, the u_c (%) shall be not greater than (≤) 15% when the concentrations of T and E are greater than (>) 5 ng/mL.~~

~~• GC-MS^n CP Analysis Requirements~~

~~- A Solid Phase Extraction (SPE) shall be performed prior to the enzymatic hydrolysis of the *Sample*;~~

~~- Calibration standard(s) and at least two (2) QC urine samples containing representative low and high levels of the *Markers* of the steroid profile shall be included.~~

~~3.2.2 GC/C/IRMS CP~~

~~Technical and reporting requirements for the GC/C/IRMS CP are specified in the TD IRMS [7].~~

~~When an *AAF* is reported for the *Marker*(s) of the steroid profile based on the results of a GC/C/IRMS analysis performed on the "A" *Sample*, only the GC/C/IRMS analysis, including the identification of the relevant *Markers* (target compounds and endogenous reference compounds) shall be repeated during the "B" *Sample* CP.~~

2.2.2.1 CP Validation Requirements (see also ISL *TD* VAL [5])

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Identification of *Markers*	The Laboratory shall validate the Qualitative Procedure for confirmation of the identity of the urinary steroid *Markers* in accordance with the requirements of the ISL *TD* IDCR [3] and ISL *TD* VAL [5].						
Maximum allowed Relative Combined Standard Measurement Uncertainty $u_{c\ Max}$ (%)	**Level**	**A**	**Etio**	**Adiols (5α-, 5β-)**	**T**	**E**	**T/E**
	at 5 x LOQ	≤ 15%					
	[T] and [E] > 5 ng/mL				≤ 15%		

2.2.2.2 CP Analysis Requirements

Quantitative Procedure	a) The concentrations of the urinary steroid *Markers* shall be confirmed in a single measurement by applying a GC-MS^n Quantitative Procedure. Appropriate isotopically-labelled preparations of urinary steroid *Marker*(s) shall be used as internal standards. b) The Analytical Method shall be capable of measuring the total content of the urinary steroid *Markers*, *i.e.*, the sum of the measured concentrations of the free steroids released after hydrolysis of their glucuronide-conjugated phase-II *Metabolites* and the free steroid fraction excreted as unconjugated. The same or different Aliquot(s) may be used for these determinations, in accordance with Test Method validation results. c) The concentration of T in the free fraction (T_{free}) shall be used to assess the validity of the *Sample* for the *ABP* (see Article 2.2.3 e). d) In each sequence of analysis, a calibration curve shall be included. e) At least one QC sample, depending on the ITP quantification results for the *Markers*, shall be included in each confirmatory analytical batch.
Qualitative Procedure	The identification of the urinary steroid *Markers* that triggered the CP shall be confirmed by a GC-MS^n Qualitative Procedure (in compliance with the ISL *TD* IDCR [3]).
Enzymatic Hydrolysis	a) An enzyme with substrate specificity for β-D-glucuronide conjugates and minimizing the risk of conversion of endogenous steroids *(e.g.*, purified β-glucuronidase from *E. coli*) shall be used for the hydrolysis of the glucuronide-conjugated urinary steroids.

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	b) An SPE procedure shall be performed prior to the enzymatic hydrolysis of the *Sample* to minimize the impact of potential microbial activity. c) The efficiency of hydrolysis shall be controlled in each Aliquot, according to CP validation data, with isotopically labeled A-glucuronide (or an equivalent scientifically recognized alternative).
Factors impacting the urinary steroid *Markers*	The Laboratory shall: a) Monitor for signs of microbial activity (*e.g.* presence of indicators of HSD activity and presence of the urinary steroid *Markers* in the free fraction). b) Test for the presence of the following non-prohibited substances: i. Conjugated *Metabolite*(s) of ethanol [*e.g.*, ethanol glucuronide (EtG)], and ii. 5α-reductase inhibitors (*e.g.*, finasteride, dutasteride), if requested.

~~3.2~~ Reporting Confirmation Procedure Results ~~from~~for the ~~CP~~

~~2.2.2~~2.2.3 ~~3.3.1 "A" *Sample*~~Urinary Steroid *Markers*

Following the ~~CP performed for the steroid profile on the "A" *Sample*~~performance of the CP, the Laboratory shall report in *ADAMS*:

a) The confirmed SG of the *Sample* ~~(determined from a new Aliquot of the "A" *Sample*);~~

~~i.~~ The confirmed ~~value~~total concentrations, expressed in ng/mL, of the urinary steroid *Markers* (*i.e.,* the combination of the ~~steroid profile (concentrations, T/E value), without adjustment for the SG~~free steroid fraction released after hydrolysis of its glucuronide-conjugated phase-II *Metabolite* and the ~~*Sample*;~~

b) ~~The~~ unconjugated free steroid) and the associated Measurement Uncertainty (u_c ~~(~~, expressed in ~~units);~~ng/mL).

[Comment to Article 2.2.3 b): If the concentration(s) cannot be determined (e.g., the estimated concentration is lower than (<) the LOQ) or the urinary steroid Marker(s) cannot be identified during the CP, the affected Marker(s) shall be reported as "-1" and the Laboratory shall make a corresponding comment in the Lab Results in ADAMS (e.g., matrix interferences).]

c) The confirmed T/E ratio (calculated from the confirmed T and E concentrations).

~~c)~~d) The GC/C/IRMS ~~confirmation~~ results~~, if~~ (as per ISL *TD* IRMS [4]), where applicable, or add a comment that the GC/C/IRMS analysis was not performed ~~(see TD IRMS [7]). The Laboratory shall update the Test Report for the *Sample* in *ADAMS* (as *AAF*,~~

~~*Atypical Finding* (*ATF*), or Negative Finding) based on the results~~at the request of the ~~GC/C/IRMS CP:~~TA.

e) The confirmed ~~results (presence/absence) for signs~~concentrations of the indicators of microbial activity~~:~~, expressed in ng/mL:

i. 5~~α~~αAND

ii. 5βAND, and

iii. T in the free fraction (T_{free}).

The ratios 5αAND/A, 5βAND/Etio~~,~~ and T_{free}/T_{total}~~; based on concentrations;~~ will be automatically calculated in *ADAMS*.

[Comment~~:~~ to Article 2.2.3 e): In addition to the determination of the 5~~α~~αAND/A and 5βAND/Etio ratios as ~~signs~~indicators of microbial contamination, the determination during the CP of an elevated ~~ratio of free Testosterone to total Testosterone (T_{free}/T_{total}~~ ratio > 0.05~~)~~ will also invalidate ~~(the steroid profile of) the Sample. However, this shall not preclude the performance of the GC/C/IRMS CP or invalidate its results~~the urine Sample for the ABP.]

~~ii.~~ The presence~~ or absence~~ in the *Sample* of substance(s) that ~~do not constitute an *AAF* but~~ may alter the urinary steroid ~~profile (see Article 1.3):~~*Markers*: if detected in the *Sample*, the Laboratory shall report the confirmed estimated ~~levels of EtG, 5α reductase inhibitors and azoles~~concentrations as specified in Article 2.~~2 (without the need to report the u_c for these determinations).~~

~~3.3.2 "B" *Sample*~~

~~Following the performance of the GC/C/IRMS CP for the steroid profile on the "B" *Sample*, the Laboratory shall report the GC/C/IRMS confirmation results (see TD IRMS [7]) in *ADAMS*.~~

~~*[Comment: If the Sample has not been reported as an AAF for the Marker(s) of the steroid profile based on the results of the GC/C/IRMS analysis, but the steroid profile CP by GC-MS^n has been requested for the "B" Sample, then the Laboratory shall report in ADAMS the results of the "B" confirmation of the steroid profile as described for the "A" Sample in Article 3.3.1.]*~~

~~2.0 Reporting *Sample* Manipulation (*Tampering* or *Attempted Tampering*)~~

~~*Tampering* or *Attempted Tampering* aims to alter the integrity and validity of *Samples* collected during *Doping Control*, including, but not limited to *Sample* substitution with another fluid and urine exchange and/or adulteration (*e.g.* addition of proteases to *Sample*).~~

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~~*[Comment: the substitution of an Athlete's urine Sample with the urine of another individual (urine exchange) can be uncovered using the steroidal Passport and confirmed by DNA analysis across multiple Samples, as described in the TD APMU [8].]*~~

~~d)~~f) ~~In cases when a *Sample* is not consistent with human urine (*e.g.* SG ≤~~ 1.~~001, creatinine ≤ 5 mg/dL [9], non-physiological salt concentration, abnormal pH values, absence or abnormally low levels of endogenous steroids, corticosteroids, proteins, etc.), the Laboratory shall:~~3.e).

~~a) Report the finding as an *AAF* for *Tampering* or *Attempted Tampering* (class M2.1 of the *Prohibited List*) if the Laboratory can determine the general nature/type of the adulterated *Sample*, which is not consistent with human urine (*e.g.* water, liquor, synthetic urine);~~

~~OR~~

~~b) Report the finding as an *ATF* for *Tampering* or *Attempted Tampering* and include a comment in *ADAMS* advising the *Testing* Authority to perform further investigations (*e.g.* additional analyses on the *Sample*, *Target Testing* the *Athlete*).~~

3.0 ~~5.0~~ References

~~[1] Mareck U *et al*. Factors influencing the steroid profile in doping control analysis. *J Mass Spectrom*. **43**(7):877-91, 2008.~~

~~[2] Ayotte C. Detecting the administration of endogenous anabolic androgenic steroids. *Handb Exp Pharmacol*. **195**:77-98, 2010.~~

~~[3] Kuuranne T, Saugy M, Baume N. Confounding factors and genetic polymorphism in the evaluation of individual steroid profiling. *Br J Sports Med*. **48**(10): 848-55, 2014.~~

~~[4]~~[1] The World Anti-Doping *Code International Standard* for ~~*Results Management*~~Laboratories.

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

~~[5]~~ *WADA Technical Document* ~~TD DL: *Decision Limits* for the Confirmatory Quantification of Exogenous~~ Threshold ~~Substances by Chromatography-based~~ Analytical ~~Methods~~.

~~[6]~~[3] ~~*WADA Technical Document*~~ISL *TD* IDCR: Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for *Doping Control* Purposes.

~~[7]~~[4] *WADA Technical Document* ISL *TD* IRMS: Detection of Synthetic Forms of *Prohibited Substances* by GC/C/IRMS.

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~~[8]~~[5] *WADA Technical Document* ISL *TD* ~~APMU: *Athlete* Passport Management Unit –~~VAL: Minimum Requirements ~~and~~for Validation of Analytical *Testing* Procedures for *Doping Control*.

~~[9] Cook J D *et al*. The Characterization of Human Urine for Specimen Validity Determination in Workplace Drug Testing: A Review. *J Anal Toxicol* **24**: 579-588, 2000~~

[6] *WADA Technical Document* ISL *TD DL*: *Decision Limits* for the Confirmatory Quantification of Exogenous Threshold Substances.

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

[Comment to Article 3.0: Current versions of WADA International Standards and Technical Documents may be found at ~~https://www.wada-ama.org/en/what-we-do/science-medical/laboratories~~ https://www.wada-ama.org/en/what-we-do/international-standards]