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Written by:	*WADA* Laboratory Expert Group	Approved by:	*WADA* Executive Committee
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Endogenous Anabolic Androgenic Steroids
Measurement and Reporting

1.0 Introduction

The purpose of this Technical Document (TD) is to harmonize the approaches to the measurement and reporting of Endogenous Anabolic Androgenic Steroids (EAAS) in urine, including data in support of the steroidal module of the *Athlete* Biological Passport (ABP) or "steroid profile".

EAAS concentrations and their ratios form the urinary "steroid profile", which may be altered following the administration of synthetic forms of EAAS, in particular testosterone (T), its precursors [for example androstenediol, androstenedione and prasterone (dehydroepiandrosterone or DHEA)], or its active metabolite [dihydrotestosterone (DHT)], as well as epitestosterone (E).

The steroid module of the ABP uses the Adaptive Model to identify an *Atypical Passport Finding* (*ATPF*), which triggers the performance of Confirmation Procedures. It is also used to apply intelligent longitudinal target *Testing* of the *Athlete*. Furthermore, an abnormal "steroid profile" (obtained from a single urine *Sample*) or an atypical "longitudinal steroid profile" (including values obtained from a series of "steroid profiles" collected over a period of time), may be a means to pursue an anti-doping rule violation (ADRV).

EAAS *Testing* and reporting follows a two-step procedure. An Initial Testing Procedure is conducted to estimate the "steroid profile" of the *Athlete's Sample*. A subsequent Confirmation Procedure is performed when the estimated "steroid profile" constitutes an *ATPF*, as determined by the Adaptive Model, or represents a "suspicious steroid profile" (SSP) finding.

The Confirmation Procedure includes the quantification of the *Markers* of the "steroid profile" as described in this TD as well as Gas Chromatography – Combustion - Isotope Ratio Mass Spectrometry (GC-C-IRMS) analysis, which is considered in a separate TD (TDIRMS) [1].

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1.1 The "Steroid Profile"

Each urine *Sample* shall be analyzed to determine its "steroid profile".

For the purposes of this TD, the "steroid profile" is composed of the following *Markers* (as free steroid content obtained from the free steroid fraction plus those released from the conjugated fraction after hydrolysis with β-glucuronidase from *E. coli*):

- androsterone (A);
- etiocholanolone (Etio);
- 5α-androstane-3α,17β-diol (5αAdiol);
- 5β-androstane-3α,17β-diol (5βAdiol);
- testosterone (T);
- epitestosterone (E).

and the following ratios:

- testosterone to epitestosterone (T/E) ;
- androsterone to testosterone (A/T);
- androsterone to etiocholanolone (A/Etio);
- 5α-androstane-3α,17β-diol to 5β-androstane-3α,17β-diol (5αAdiol/5βAdiol); and
- 5α-androstane-3α,17β-diol to epitestosterone (5αAdiol/E).

The administration of EAAS can alter one or more of the *Markers* and/or ratios of the urinary "steroid profile", resulting in increase or decrease of concentrations and/or ratios of specific pairs of steroid *Metabolites* [2-4].

Additionally, alteration of the urinary "steroid profile" can occur for a number of reasons including, but not limited to:

- the administration of other anabolic steroids (*e.g.* stanozolol);
- the administration of human chorionic gonadotrophin (hCG) in males;
- the administration of inhibitors of 5α-reductase (*e.g.* finasteride);
- a large intake of alcohol (ethanol);
- the administration of ketoconazole or other similar compounds;the use of masking agents (*e.g.* probenecid) and diuretics; or
- microbial growth.

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2.0 Initial Testing Procedure

The Laboratory shall use a validated Initial Testing Procedure that is fit-for-purpose to estimate the *Markers* of the urinary "steroid profile" in the range of values determined in males and females.

The Initial Testing Procedure is conducted on a single Aliquot.

2.1 Method Characteristics

- Gas chromatography combined with mass spectrometry (GC-MS or GC-MS/MS) of TMS derivatives (keto and hydroxyl groups) is required.
- Calibration standard(s) or a calibration curve should be included in each sequence of analysis.
- At least two urine quality control (QC) samples containing low and high representative concentrations of the *Markers* of the "steroid profile" should be included in each sequence of analysis.
- The enzymatic hydrolysis shall be carried out with purified β-glucuronidase from *E. coli* (*H. pomatia* mixtures are not acceptable).
- The completeness of hydrolysis of the glucuroconjugated urinary steroids shall be controlled with isotopically labeled A-glucuronide (or an equivalent scientifically recognized alternative).
- The completeness of the derivatization shall be controlled through the monitoring of mono-O-TMS vs. di-O-TMS derivative of A.
- When needed, the volume [1] of the *Sample* Aliquot may be adjusted as a function of its specific gravity (SG) and of the sex of the *Athlete*.
- The T/E ratios shall be determined from the ratios of the corrected chromatographic peak areas or peak heights [2].

[1] Much smaller concentrations of T and E are generally present in *Samples* from females and in those *Samples* with low SG; therefore, larger Aliquot volumes may be required for a reliable measurement.

[2] Ratios of T and E peak heights or peak areas corrected against a calibrator or a calibration curve (same mass or same ion transition screened for both steroids).

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- The linearity of the method, established during method validation, shall cover the ranges of *Marker* concentrations normally found in males and females - the limit of quantification (LOQ) for T and E shall not be greater than 2 ng/mL [3].
- The relative standard combined Measurement Uncertainty [u_c (%)] for the determination of A, Etio, 5αAdiol, 5βAdiol, T and E, as estimated during method validation of the Initial Testing Procedure, shall be not greater than 30% at the respective LOQ;
    - For concentrations at five times the LOQ, the u_c (%) shall be not greater than 20% for A and Etio or 25% for the Adiols;
    - The u_c (%) for determinations of T and E shall not exceed 20% when the steroid concentrations are greater than 5 ng/mL;
    - The u_c (%) for determinations of T/E ratios calculated from the corrected chromatographic peak areas or heights shall not exceed 15% when the concentrations of T and E are both greater than 5 ng/mL; for smaller concentrations of T or E, the u_c (%) for the T/E determinations shall not exceed 30%.
- Evidence of microbial degradation [*e.g.* presence of 5α-androstanedione (5αAND) and 5β-androstanedione (5βAND)] and the presence of 5α-reductase inhibitors (*e.g.* finasteride), ethanol *Metabolite(s)* and ketoconazole (and similar substances) shall be monitored.

[3] The LOQ shall be determined as the smallest concentration that can be measured with the uncertainty criterion established for the given *Marker* of the "steroid profile" when applying the Initial Testing Procedure.

The LOQ for T, E, A, Etio, 5αAdiol and 5βAdiol shall be recorded in *ADAMS* by the Laboratory. The LOQ values shall be updated in *ADAMS* whenever a significant change is made to the analytical method.

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2.2. Reporting the "steroid profile" from the Initial Testing Procedure

Following the performance of the Initial Testing Procedure, the Laboratory shall report the "steroid profile" of the *Sample* in *ADAMS*, including:

- the SG of the *Sample*;
- the concentrations of T, E (see Table 1), A, Etio, 5αAdiol and 5βAdiol (without adjustment for the SG of the *Sample*) [4, 5];
- the T/E ratio (see Table 1) [6];
- the results of screening for signs of microbial contamination (*e.g.* ratio of 5α-androstanedione to androsterone - 5αAND/A; ratio of 5β-androstanedione to etiocholanolone - 5βAND/Etio)[7];
- the presence or absence in the *Sample* of substance(s) that may alter the "steroid profile" [7]; and
- the validity of the "steroid profile" of the *Sample* as "Yes" or "No".

[4] When reporting the "steroid profile" in *ADAMS*, the Laboratory shall report the values of concentrations for T, E, A, Etio, 5αAdiol and 5βAdiol, and the T/E ratio (without adjustment for the urine SG or correction to a specific number of significant figures). An automatic correction of reported values to 2 significant figures will be made in *ADAMS* upon application of the Adaptive Model of the ABP to the "longitudinal steroid profile" of the *Athlete*.

[5] Any concentration measurement which is below the LOQ of the assay shall be reported as "-1" by the Laboratory. When the chromatographic peak signal for E cannot be detected (*i.e.* below the detection capability of the assay), the concentration of E shall be reported as "-2" (see Table 1).

[6] In *ADAMS*, the values of the other four ratios (A/T, A/Etio, 5αAdiol/5βAdiol and 5αAdiol/E) are automatically computed after the reporting of the "steroid profile" by the Laboratory.

[7] A *Sample* showing signs of microbial degradation or containing any of the substances that may cause an alteration of the "steroid profile" may not be suitable for inclusion in the "longitudinal steroid profile". These findings are to be considered by the *Athlete* Passport Management Unit (APMU) during the results management process when evaluating the analytical data for the *Sample* and assessing the possible pathological or confounding conditions that may have impacted an *Athlete's* analytical results.

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In cases when the Laboratory analyzes two (2) or more *Samples*, which are linked to a single *Sample* collection session from the same *Athlete*, the Laboratory shall report the "steroid profile" for each of the *Samples* analyzed.

If, as determined during the Initial Testing Procedure, no *Prohibited Substance* or *Method* is detected in the *Sample*, the Laboratory shall report the "steroid profile" of the *Sample* in *ADAMS*, while reporting the test results as "No *Prohibited Substance(s)* or *Metabolite(s)* or *Marker(s)* of a *Prohibited Method(s)* on the test menu were detected".

If, on the other hand, the Laboratory confirms the presence of a *Prohibited Substance* or *Method*, the Laboratory shall still report the "steroid profile" of the *Sample* in *ADAMS* as determined during the Initial Testing Procedure, while reporting the *Sample* as an *Adverse Analytical Finding* (or *Atypical Finding*, as applicable) for the *Prohibited Substance* or *Method* detected.

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2.2.1 Validity of (the "steroid profile" of) the *Sample*

The validity of the *Sample* shall be reported in ADAMS as "Yes" or "No".

The Laboratory shall report the validity of the *Sample* as:

a) **"No"**: **only when the *Sample* shows signs of extensive degradation**, as determined by:

- 5αAND/A ≥ 0.1 and/or 5βAND/Etio ≥ 0.1.

b) "**Yes"**: **in all other situations**, including:

- When the concentration of either T and/or E is below the Laboratory's LOQ, but its chromatographic peak signal is still measurable and the T/E ratio can be determined from the corrected chromatographic peak areas or peak heights [2]. The calculated value of the T/E ratio shall be reported in *ADAMS* whereas the concentration of T and/or E, as applicable, shall be reported as "-1" (Table 1) [5].
- When the T/E ratio cannot be determined from the ratios of the corrected chromatographic peak areas or peak heights [2] because the chromatographic peak signal for T and/or E is not detectable *(i.e.* it is below the Limit of Detection – LOD - of the assay) [8]:
  - If the chromatographic peak signal for T cannot be detected, the concentration of T and the T/E value shall be reported as "-1" (Table 1). A comment shall be included in the Test Report in *ADAMS* stating that the T/E ratio could not be measured because the concentration of T was below the detection capability of the assay;
  - If the chromatographic peak signal for E cannot be detected, the concentration of E shall be reported as "-2" and the T/E ratio shall be calculated on the basis of the Laboratory's LOQ value for E (*e.g.* if T concentration is 6 ng/mL while E cannot be detected, and the Laboratory's LOQ for E is 1.5 ng/mL, the T/E shall be reported as 4.0) (Table 1). A comment shall be included in the Test Report in

[8] When the measurement of a *Marker* of the "steroid profile" is not possible due to, for example, dilution, unusual matrix interferences, inhibition of the enzymatic hydrolysis or incomplete derivatization, the Laboratory should repeat the analysis with an alternative, validated *Sample* preparation procedure (*e.g.* solid phase extraction, extraction with a different solvent or other equivalent procedure).

ADAMS stating that the T/E ratio could not be measured accurately because the concentration of E was below the detection capability of the assay;

- If the chromatographic peak signals for both T and E cannot be detected, the concentration of T and the T/E value shall be reported as "-1", whereas the concentration of E shall be reported as "-2" (Table 1). A comment shall be included in the Test Report in *ADAMS* stating that the T/E ratio could not be measured because the concentrations of T and E were below the detection capability of the assay.

- When other *Marker*(s) of the "steroid profile" cannot be measured accurately (*i.e.* concentrations below the LOQ of the assay) [8]. In such cases, the concentration of the negatively impacted *Marker*(s) shall be reported as "-1" [5] while the validity of the *Sample* shall be reported as "Yes".

- Less extensive microbial contamination shall be reported in *ADAMS*, while the validity of the *Sample* shall be reported as "Yes" [7]:
  - 5αAND/A ratio and/or between 0.05 and 0.1,
  - 5βAND/Etio ratio between 0.05 and 0.1.

- When the Laboratory reports an *Adverse Analytical Finding* or an *Atypical Finding* for a *Prohibited Substance* that may alter the "steroid profile" (*e.g.* an anabolic steroid, hCG in males, a diuretic or masking agent)[7].

- When the Laboratory detects the presence in the *Sample* of other substances that may cause an alteration of the "steroid profile" [7, 9] .

[9] It is mandatory that the Laboratory tests at least for the presence of conjugated *Metabolite(s)* of ethanol [*e.g.* ethanol glucuronide (EtG)], inhibitors of 5α-reductase and ketoconazole during the Initial Testing Procedure and report the estimated concentration of EtG if above 5 μg/mL (without the need to report the Measurement Uncertainty).

Furthermore, the analysis of these substances shall also be included in the Confirmation Procedure of atypical or suspicious "steroid profile" findings.

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Table 1. Summary of conditions for reporting T and E concentrations and T/E ratio.

Concentration of T	Concentration of E	T/E ratio
Chromatographic peak signal of T measured at or above the LOQ. $[T] \geq LOQ_{(T)}$ **Report T as measured**	Chromatographic peak signal of E measured at or above LOQ. $[E] \geq LOQ_{(E)}$ **Report E as measured**.	**Report T/E as determined** from corrected peak heights/areas
	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 T/LOQ$_{(E)}$** *Comment in Test Report*: T/E ratio could not be measured accurately because the concentration of E was below the detection capability of the assay
Chromatographic peak signal of T detected, but below the LOQ. $LOD_{(T)} \leq [T] < LOQ_{(T)}$ **Report T as "-1"**	Chromatographic peak signal of E measured at or above LOQ. $[E] \geq LOQ_{(E)}$ **Report E as measured**	**Report T/E as measured** from corrected peak heights/areas
	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 Test Report*: T/E ratio could not be measured accurately because the concentrations of T and E could not be measured
Chromatographic peak signal of T not detected. $[T] < LOD_{(T)}$ **Report T as "-1"**	Chromatographic peak signal of E measured at or above LOQ. $[E] \geq LOQ_{(E)}$ **Report E as measured**	**Report T/E as "-1"** *Comment in Test Report*: T/E ratio could not be measured accurately because the concentration of T was below the detection capability of the assay
	Chromatographic peak signal of E detected but below LOQ. $LOD_{(E)} \leq [E] < LOQ_{(E)}$ **Report E as "-1"**	**Report T/E as "-1"** *Comment in Test Report*: T/E ratio could not be measured accurately because the concentrations of T and E could not be measured
	Chromatographic peak signal of E not detected. $[E] < LOD_{(E)}$ **Report E as "-2"**	**Report T/E as "-1"** *Comment in Test Report*: T/E ratio could not be measured accurately because the concentrations of T and E were below the detection capability of the assay

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3.0 Confirmation Procedures

Confirmation Procedures for the exogenous administration of EAAS include the GC-MS or GC-MS/MS quantification and GC-C-IRMS analysis of the relevant *Marker(s)* of the "steroid profile". GC-C-IRMS analysis is considered in a separate Technical Document, the TDIRMS [1].

"*ATPF* Confirmation Procedure Request"

Following the reporting by the Laboratory of the *Sample's* "steroid profile" in *ADAMS*, the Adaptive Model will generate an "*ATPF* Confirmation Procedure Request" notification when the following criteria are met:

1) The *Sample* is matched with a Doping Control Form (DCF) in *ADAMS*, allowing the automatic inclusion of the *Sample's* "steroid profile" in the *Athlete's* steroidal passport,
2) There is an existing "longitudinal steroid profile" of the *Athlete* in *ADAMS*,
3) The *Sample's* T/E ratio is abnormal, as determined by the Adaptive Model, when compared with the previous longitudinal T/E values of the *Athlete*.
   - Upon reception of the "*ATPF* Confirmation Procedure Request" notification for an abnormal T/E ratio through *ADAMS*, the Laboratory shall confirm T, E [10] and the T/E ratio by GC-MS or GC-MS/MS and analyze the *Markers* of the "steroid profile" by GC-C-IRMS (refer to the TD IRMS [1]).
   - The Adaptive Model will also determine abnormal values of the other ratios of the "steroid profile" (A/T, A/Etio, 5αAdiol/5βAdiol, 5αAdiol/E). However, in such cases the Laboratory will not receive an automatic "*ATPF* Confirmation Procedure Request" notification through *ADAMS*. Instead, the *Athlete* Passport Management Unit (APMU) will advise the *Testing* Authority on whether the *Sample* shall be subjected to Confirmation Procedures. Therefore, in these cases the Laboratory shall receive a request from the *Testing* Authority before proceeding with the Confirmation Procedure(s) [11].

[10] For T/E values, only T needs to be confirmed if the concentration levels of E or the volume of the *Sample* are not sufficient.

[11] Or as covered by agreement between the Laboratory and the *Testing* Authority.

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"Suspicious Steroid Profile Confirmation Procedure Request"

The Laboratory will receive a "Suspicious Steroid Profile Confirmation Procedure Request" notification through *ADAMS* if:

1) The *Sample* is matched with a DCF in *ADAMS*, but there is no existing "longitudinal steroid profile" of the *Athlete* in *ADAMS* (*i.e.* this is the first *Sample* in the *Athlete's* steroidal passport), or

   The *Sample* cannot be matched with a DCF in *ADAMS* within fourteen (14) calendar days after the reception date of the *Sampl*e by the Laboratory, and therefore the "steroid profile" of the *Sample* cannot be processed by the Adaptive Model in *ADAMS*,

   and

2) The *Sample's* "steroid profile" meets **any** of the following criteria:
   - T/E ratio (calculated from the corrected chromatographic peak areas or heights) greater than 4.0;
   - A/T ratio less than 20;
   - 5αAdiol/5βAdiol ratio greater than 2.4;
   - concentration of T or E (adjusted for the SG[12]) greater than 200 ng/mL in males or greater than 50 ng/mL in females;
   - concentration of A or Etio (adjusted for the SG[12]) greater than 10,000 ng/mL;
   - concentration of 5αAdiol (adjusted for the SG[12]) greater than 250 ng/mL in males or greater than 150 ng/mL in females, combined with a 5αAdiol/E ratio greater than 10 in either sex.

- Upon receipt of the "Suspicious Steroid Profile Confirmation Procedure Request" notification, the Laboratory shall proceed with the Confirmation Procedure(s) unless, after contacting the *Testing* Authority, the *Testing* Authority can justify in writing within seven (7) calendar days that the Confirmation Procedure(s) is not necessary. Justification for not proceeding with the Confirmation Procedure may

[12] The concentrations are adjusted to a urine SG of 1.020 based on the following equation (free and hydrolyzed glucuroconjugated steroids).

$$\mathbf{Conc_{corr} = Conc_{measured} * (1.020 - 1)/(SG - 1)}$$

include, for example, a naturally elevated T/E ratio confirmed by previous *Testing*, or a T/E ratio between 4.0 and 6.0 for the first test on the *Athlete*.

- If the *Testing* Authority justifies that confirmation is not necessary, the Laboratory shall update the *ADAMS* report for the *Sample* with a comment stating that the *Testing* Authority considered that the Confirmation Procedure(s) was not necessary and the explanation provided by the *Testing* Authority. If the *Testing* Authority cannot justify that confirmation is not necessary, the Laboratory shall proceed with the confirmation analyses.
- In cases when the Laboratory receives "*ATPF* Confirmation Procedure Requests" or "Suspicious Steroid Profile Confirmation Procedure Requests" for two (2) or more *Samples*, which are linked to a single *Sample* collection session from the same *Athlete*, the Laboratory, in consultation with the *Testing* Authority, shall prioritize the confirmation of the *Sample* with the highest concentration levels of the *Markers* of the "steroid profile".
- When the Laboratory receives an "*ATPF* Confirmation Procedure Request" or a "Suspicious Steroid Profile Confirmation Procedure Request" for a *Sample* for which *Adverse Analytical Finding(s)* have been reported for other *Prohibited Substance(s)* or *Method(s)*, the Laboratory should consult the *Testing* Authority about the need to conduct the Confirmation Procedures for the *Markers* of the "steroid profile".
- A Laboratory may have a contractual agreement in place with the *Testing* Authority to conduct the Confirmation Procedures when a *Sample* meets any of the analytical criteria of a "suspicious steroid profile" or at the Laboratory's discretion based on its expertise.

  Under such circumstances, the Laboratory may proceed to the confirmation of the "suspicious steroid profile" immediately without waiting for an "*ATPF* Confirmation Procedure Request" or a "Suspicious Steroid Profile Confirmation Procedure" request from *ADAMS.* Following the performance of the Confirmation Procedure(s), the Laboratory shall report in *ADAMS* the "steroid profile" of the *Sample* as determined during the Initial Testing Procedure as well as the confirmed values of the *Markers* of the "steroid profile" and the GC-C-IRMS test results. Furthermore, the Laboratory shall report the *Sample*

test result in *ADAMS* (as *Adverse Analytical Finding*, *Atypical Finding*, or "No *Prohibited Substance*(s) or *Metabolite*(s) or *Marker*(s) of a *Prohibited Method*(s) on the test menu were detected") based on the results of the GC-C-IRMS Confirmation Procedure in accordance with the TDIRMS [1].

3.1 GC-MS or GC-MS/MS quantification Confirmation Procedure

The Laboratory shall identify (in compliance with the TDIDCR [5]) and quantify the relevant *Markers* of an *ATPF* or a SSP finding in one additional *Sample* Aliquot by a validated fit-for-purpose GC-MS or GC-MS/MS quantification method.

- The Laboratory shall confirm the abnormal *Markers* (concentrations, T/E*)* of the "steroid profile" that triggered the *ATPF* or SSP finding before proceeding with the GC-C-IRMS analysis [10, 13].
- If a GC-C-IRMS analysis is to be performed on a *Sample* with a normal "steroid profile" upon request from the *Testing* Authority, the *Athlete* Passport Management Unit (APMU), or *WADA*, the Laboratory shall consult with the relevant authority to determine which *Marker(s)* of the "steroid profile" require quantification.

During the Confirmation Procedure, the presence of conjugated *Metabolite(s)* of ethanol (*e.g* EtG), inhibitors of 5α-reductase (*e.g.* finasteride), ketoconazole as well as the signs of microbial degradation including, for example, the presence of the free forms of T, 5αAND or 5βAND, shall be determined.

[13] Upon reception of the immediate "*ATPF* Confirmation Procedure Request" notification for an abnormal T/E ratio through *ADAMS*, the Laboratory shall confirm the concentrations of T and E [10], and the T/E ratio.

- In cases of abnormal findings for other ratios of the "steroid profile", the Laboratory shall confirm the relevant concentrations of the *Markers* of the "steroid profile" upon request from the *Testing* Authority [11].

In cases of "Suspicious Steroid Profile Confirmation Procedure Requests", the Laboratory shall confirm the relevant concentrations of the *Markers* of the "steroid profile", which produced the suspicious finding, and the T/E ratio, if applicable (T/E > 4.0), in consultation with the *Testing* Authority.

3.1.1 Method Characteristics for GC-MS or GC-MS/MS quantification Confirmation Procedure

The same analytical requirements presented in 2.1 apply, with the following modifications:

- Calibration standards and urine QC samples containing representative levels of the *Markers* of the "steroid profile" shall be included.
- The u_c (%) shall be not greater than 15% for determinations of A, Etio, 5αAdiol and 5βAdiol at concentrations representing five times 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.

3.2 Reporting Results from the Confirmation Procedures

Following the performance of the Confirmation Procedure(s) on the "A" or the "B" *Sample* [14], the Laboratory shall report in *ADAMS*:

- the SG of the *Sample;*
- the confirmed values (*e.g.* concentrations, T/E ratio) of the *Markers* of the "steroid profile", without adjustment for the SG of the *Sample* (Table 1)[5, 6];
- the associated u_c expressed in units;
- the GC-C-IRMS confirmation results (refer to TD IRMS [1]) [14];
- the confirmed results for signs of microbial contamination (*e.g.* 5αAND/A, 5βAND/Etio, T_{free} / T_{total} [15]);

[14] When an *Adverse Analytical Finding* 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 shall be repeated during the "B" *Sample* Confirmation Procedure, if applicable. Refer to the TDIRMS [1].

[15] In addition to the determination of the 5αAND/A and 5βAND/Etio ratios as signs of microbial contamination, as described in section 2.2.1 for the Initial Testing Procedure, the determination during the Confirmation Procedure of an elevated ratio of free Testosterone to total Testosterone (T_{free} / T_{total} > 0.05) shall also invalidate (the "steroid profile" of) the *Sample.*

- the validity of the *Sample* (as per section 2.2.1 above)[15, 16];
- the confirmed presence of conjugated *Metabolite(s)* of ethanol, inhibitors of 5α-reductase (*e.g.* finasteride), ketoconazole or any other substances that might have altered the "steroid profile", if applicable. The Laboratory shall report the confirmed estimated levels of EtG if above 5 μg/mL (without the need to report the Measurement Uncertainty for this determination).

Following the confirmation of an *ATPF* or SSP, the Laboratory shall update the *ADAMS* test result record for the *Sample* (as *Adverse Analytical Finding*, *Atypical Finding*, or No *Prohibited Substance*(s) or *Metabolite*(s) or *Marker*(s) of a *Prohibited Method*(s) on the test menu were detected) based on the results of the GC-C-IRMS Confirmation Procedure in accordance with the TDIRMS [1]).

3.3 Additional Analyses: Steroid Ester(s) and DNA

When matched blood *Samples* have been collected during the same *Sample* Collection Session as urine *Samples* identified with an atypical or suspicious "steroid profile", Laboratories, in consultation with the *Testing* Authority, should consider conducting analysis to detect the presence of steroid ester(s) in serum/plasma.

It is recommended that confirmation analyses for steroid ester(s) serum/plasma be conducted prior to the performance of the GC-C-IRMS analysis in urine. The detection of steroid ester(s) in serum/plasma also constitutes an unequivocal demonstration of the exogenous origin of the steroid(s). On the other hand, the absence of detectable steroid ester(s) in serum/plasma does not invalidate a GC-C-IRMS positive result in urine.

The performance of DNA analyses may also be considered to establish, in conjunction with the *Athlete's* "longitudinal steroid profile", the individual origin of the *Sample*(s).

[16] The reporting of the validity of the *Sample* shall not be based on the results of the GC-C-IRMS confirmation analysis.

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4.0 References

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