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].
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.
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 \(^2\).

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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).
• 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;
  o 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;
  o The \(u_c(\%)\) for determinations of T and E shall not exceed 20% when the steroid concentrations are greater than 5 ng/mL;
  o 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.

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\(^3\) 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.
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-androstane to androsterone - 5αAND/A; ratio of 5β-androstane 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.
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.
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:
   - $5\alpha\text{AND/A} \geq 0.1$ and/or $5\beta\text{AND/Etio} \geq 0.1$.

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. 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).

- When the T/E ratio cannot be determined from the ratios of the corrected chromatographic peak areas or peak heights 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):
  - 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 ADAMS stating that the T/E ratio could not be measured because the concentration of E was below the detection capability of the assay.

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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;
o 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). In such cases, the concentration of the negatively impacted Marker(s) shall be reported as “-1” 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”:
  o 5αAND/A ratio and/or between 0.05 and 0.1,
  o 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).

- When the Laboratory detects the presence in the Sample of other substances that may cause an alteration of the “steroid profile”.

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

<table>
<thead>
<tr>
<th>Concentration of T</th>
<th>Concentration of E</th>
<th>T/E ratio</th>
</tr>
</thead>
</table>
| Chromatographic peak signal of T measured at or above the LOQ. | [E] ≥ LOQ(E)  
Report E as measured. | Report T/E as determined from corrected peak heights/areas |
| [T] ≥ LOQ(T)  
Report T as measured | LOD(E) ≤ [E] < LOQ(E)  
Report E as “-1” | |
| Chromatographic peak signal of E measured at or above LOQ. | | |
| LOD(E) ≤ [E] < LOQ(E)  
Report E as “-2” | | |
| Chromatographic peak signal of T detected, but below the LOQ. | Report T as “-1”  
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 | |
| LOD(T) ≤ [T] < LOQ(T)  
Report T as “-1” | 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 E detected, but below LOQ. | | |
| [E] < LOD(E)  
Report E as “-2” | | |
| Chromatographic peak signal of T not detected. | Report T 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 | |
| [T] < LOD(T)  
Report T as “-1” | 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) ≤ [E] < LOQ(E)  
Report E as “-1” | | |
| Chromatographic peak signal of T not detected. | Report T 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 | |
| [T] < LOD(T)  
Report T 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 were below the detection capability of the assay | |
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) $^{11}$.

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$^{10}$ 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.
“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 Sample 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:
   o T/E ratio (calculated from the corrected chromatographic peak areas or heights) greater than 4.0;
   o A/T ratio less than 20;
   o 5αAdiol/5βAdiol ratio greater than 2.4;
   o concentration of T or E (adjusted for the SG\textsuperscript{12}) greater than 200 ng/mL in males or greater than 50 ng/mL in females;
   o concentration of A or Etio (adjusted for the SG\textsuperscript{12}) greater than 10,000 ng/mL;
   o concentration of 5αAdiol (adjusted for the SG\textsuperscript{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

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

\[ \text{Conc}_{\text{corr}} = \text{Conc}_{\text{measured}} \times \frac{(1.020 - 1)}{(SG - 1)} \]
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
3.1 GC-MS or GC-MS/MS quantification Confirmation Procedure

The Laboratory shall identify (in compliance with the TDIDC [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, $5\alpha$Adiol and $5\beta$Adiol 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\textsuperscript{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)\textsuperscript{5, 6};
- the associated $u_c$ expressed in units;
- the GC-C-IRMS confirmation results (refer to TD IRMS [1])\textsuperscript{14};
- the confirmed results for signs of microbial contamination (e.g. $5\alpha$AND/A, $5\beta$AND/Etio, $T_{\text{free}} / T_{\text{total}}$\textsuperscript{15}).

\textsuperscript{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 TD IRMS [1].

\textsuperscript{15} In addition to the determination of the $5\alpha$AND/A and $5\beta$AND/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 ($T_{\text{free}} / T_{\text{total}} > 0.05$) shall also invalidate (the “steroid profile” of) the Sample.
• the validity of the Sample (as per section 2.2.1 above)\textsuperscript{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).

\textsuperscript{16} The reporting of the validity of the Sample shall not be based on the results of the GC-C-IRMS confirmation analysis.
4.0 References

1. WADA Technical Document TDIRMS (current version): Detection of synthetic forms of Endogenous Anabolic Androgenic Steroids by GC-C-IRMS. 


