



## **TD2016EAAS v1.0**

### **Summary of Major Modifications**

The Technical Document on Measuring and Reporting of Endogenous Anabolic Androgenic Steroids has undergone a further revision by WADA's Laboratory Expert Group (LabEG). The modified document, TD2016EAAS v1.0, includes important changes in regards to the reporting of the values (concentrations, T/E ratio), the validity of measured steroid profiles and the conditions that trigger the performance of GC-MS<sup>n</sup> and GC-C-IRMS confirmation analyses by the anti-doping laboratories.

#### **1.1 The "Steroid Profile"**

- Four additional ratios (A/T, A/Etio, 5 $\alpha$ Adiol/5 $\beta$ Adiol, 5 $\alpha$ Adiol/E) are now included as longitudinal markers of the "steroid profile".
- The list of the relevant confounding factors has been reorganized.
- Footnote 1 has been removed. The same information about the automatic calculation of these four ratios in ADAMS is provided in footnote 6.

#### **2.1 Method Characteristics**

- It has been specified that the laboratory should implement at least two urine quality control (QC) samples, containing low and high representative concentrations of the markers of the "steroid profile", in each sequence of analysis.
- In the last bullet point, it is specified that, in addition to microbial degradation and the presence of 5 $\alpha$ -reductase inhibitors, the laboratory shall also monitor the presence of ethanol metabolites and ketoconazole (and similar substances) during the Initial Testing Procedure. In the previous version of the document (TD2014EAAS v2.0), testing for these two confounding factors during the Initial Testing Procedure was not mandatory.

#### **2.2 Reporting the 'steroid profile' from the Initial Testing Procedure**

- For better clarity, the requirements for reporting the "steroid profile" are formatted as a bullet-point list. It is clarified that the laboratory shall test for the presence of confounding factors (*e.g.* microbial contamination or any other factor that may alter the profile) during the Initial Testing Procedure and shall report the results.
- A new paragraph has been introduced emphasizing that the laboratory shall report the "steroid profile" for each sample analyzed, even in cases when 2 or more samples are linked to a single sample collection session from the same athlete.
- Detailed instructions are provided on the reporting of test results, including the "steroid profile" as determined during the Initial Testing Procedure, in cases when

the sample does not contain any prohibited substance or when a prohibited substance or method has been confirmed.

- Footnote 5 includes an important change regarding the reporting of the Epitestosterone (E) concentration as “-2” when the chromatographic signal for this steroid is below the limit of detection (LOD) of the assay. Reference is made to the newly introduced Table 1 (page 9), which summarizes the conditions for reporting T and E concentrations as well as the T/E ratio.
- In line with the changes implemented regarding the reporting of sample validity (see below), footnote 6 specifies that any findings associated with the presence of a confounding factor are to be considered by the Athlete Passport Management Unit (APMU) when evaluating the analytical data for the sample.

### **2.2.1 Validity of (the “steroid profile” of) the sample**

- This new subparagraph has been created to address the significant changes implemented on the reporting of sample validity.
- It is specified that the sample shall be considered invalid (validity reported as “no”) only when there are signs of extensive degradation. The criteria to establish such degradation are provided.
- For any other situation, including those circumstances when the T/E ratio cannot be measured reliably, when other markers of the “steroid profile” cannot be quantified, when there are signs of non-extensive microbial degradation or when confounding factors are detected in the sample, the sample shall be reported as valid. “Steroid profile” findings associated with the presence of a confounding factor are to be considered by the Athlete Passport Management Unit (APMU) when evaluating the analytical data for the sample.
- Footnote 9 emphasizes that it is mandatory that the laboratory tests for the presence of conjugated metabolite(s) of ethanol, inhibitors of 5 $\alpha$ -reductase and ketoconazole during the Initial Testing Procedure and report the estimated concentration of ethyl glucuronide if above 5  $\mu$ g/mL.
- Table 1 (page 9) summarizes the different conditions for reporting T and E concentrations and the T/E ratio.

### **3.0 Confirmation Procedure**

Further provisions are provided on the conditions that shall trigger IRMS and GC-MS<sup>n</sup> confirmation analyses for the steroid profile.

- “Atypical passport Finding (ATPF) Confirmation Procedure Request”: ATPFs are automatically triggered only when the Adaptive Model determines that the T/E ratio of the sample is abnormal. Therefore, population-based criteria (e.g. T > 200 ng/mL in males) have been removed and are considered only as part of a “Suspicious Steroid Profile”. The Adaptive Model will also determine abnormal values of the other ratios of the “steroid profile” (A/T, A/Etio, 5 $\alpha$ Adiol/5 $\beta$ Adiol, 5 $\alpha$ Adiol/E), but in such cases the decision on performing confirmation analyses will be taken by the Testing Authority (following advice by the APMU).
- “Suspicious Steroid Profile (SSP) Confirmation Procedure Request”: The population-based criteria that will trigger a SSP finding have been expanded to include specific requirements for the A/T ratio, the concentrations of A and Etio, as well as the concentration of 5 $\alpha$ Adiol in combination with the 5 $\alpha$ Adiol/E ratio.

- Examples are provided of possible justifications that could be given by Testing Authorities for not proceeding with the confirmation of a SSP finding.
- A new paragraph has been introduced specifying that, in cases of ATPF or SSP findings for 2 or more samples linked to a single sample collection session from the same athlete, the Laboratory shall consult the Testing Authority to prioritize the confirmation of the sample with the highest concentration levels of the markers of the "steroid profile".
- A new paragraph has been introduced specifying that the laboratory shall consult with the Testing Authority regarding the need to confirm the "steroid profile" of a sample for which Adverse Analytical Finding(s) (for other prohibited substance(s) or method(s)) have already been reported.
- Instructions are given on the immediate confirmation and reporting of the "steroid profile" when a laboratory has a contractual agreement in place with the Testing Authority to confirm any "suspicious steroid profile" based on the Laboratory's expertise.

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

- It has been specified that the Laboratory shall first 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. Footnote 13 has been introduced to provide further guidance.
- It is also established that the Laboratory shall consult with the Testing Authority to clarify which markers of the "steroid profile" require confirmatory quantification in cases when GC-C-IRMS analysis is requested on a sample with a normal "steroid profile".

### **3.2 Reporting Results from the Confirmation Procedures**

- Criteria are provided on how to report the "steroid profile" after conducting the confirmation procedure(s) on the "A" or the "B" sample. In addition to the markers of the profile, the Laboratory shall confirm the test results on the presence of the confounding factors (e.g. microbial contamination, conjugated metabolite(s) of ethanol, inhibitors of 5 $\alpha$ -reductase, etc.) obtained from the Initial Testing Procedure.
- Instructions are provided for updating the sample's ADAMS test result record based on the results of the GC-C-IRMS confirmation procedure. The reporting of the validity of the sample shall not be based on the results of the GC-C-IRMS confirmation analysis (footnote 16).
- Footnote 14 has been added to clarify that 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, the "B" sample confirmation procedure only requires that the GC-C-IRMS analysis be performed.

### **3.3 Additional Analyses: Steroid Ester(s) and DNA**

This new paragraph describes the analysis of steroid esters in serum/plasma as an alternative confirmation test to unequivocally establish the exogenous origin of the steroids, as well as the application of DNA analyses, in limited situations, to determine the individual origin of samples.