



## **TD2016IRMS**

### ***Summary of Major Modifications***

The Technical Document on Detection of Synthetic Forms of Endogenous Anabolic Androgenic Steroids by GC-C-IRMS has undergone a further revision by WADA's Laboratory Expert Group (LabEG). The modified document, TD2016IRMS v1.0, includes important changes in regards to the analytical requirements, interpretation and reporting of GC-C-IRMS results.

#### **1.1 Application of GC-C-IRMS**

This section has been divided into two subsections to specifically address the use of GC-C-IRMS analysis:

1.1.1 as a confirmation procedure to establish the exogenous administration of EAAS (*i.e.* the administration of synthetic forms of testosterone or related steroids) which constitute the markers of the "steroid profile"), and

1.1.2 to establish the possible exogenous origin of formestane, boldenone or boldenone metabolites.

#### **1.1.2 GC-C-IRMS analysis for formestane, boldenone or boldenone metabolite(s)**

This subparagraph has been edited to address the different conditions that shall trigger the GC-C-IRMS analysis for formestane, boldenone or boldenone metabolite(s). Instructions are provided on how to interpret the findings in circumstances when, as determined by the concentration of the analyte(s) in the sample, the GC-C-IRMS analysis is not considered necessary.

#### **2.0 GC-C-IRMS analysis**

- It has been clarified that the target compounds covered in this technical document are the markers of the "steroid profile" and non-threshold substances such as boldenone, boldenone metabolites and formestane.
- The four endogenous compounds that can be used as endogenous reference compound have been specified.

#### **2.1 GC-C-IRMS Method Characteristics**

- In this section, the GC-C-IRMS method performance characteristics are specified with the objective of further harmonizing assay performance across WADA-accredited laboratories.

- New specifications are given on the determination of the linearity of the ion source, instrumental linearity, method linearity and limits of quantification (LOQ) as part of the method validation.
- In addition, clarification is provided on the measurement of a minimum 40 volunteer urine samples and/or athlete negative samples to evaluate method performance by the laboratory according to specific criteria.
- It is required to subject the negative and positive urine quality control (QC) samples to the same sample preparation procedure (including hydrolysis of glucuronide conjugates and analyte derivatization, if applicable) as the test samples, and the consistency of the  $\delta^{13}\text{C}$  determinations of the QC samples shall be monitored through the use of QC-charts.
- It is specified that the reference material shall contain the relevant free (unconjugated) steroids, underivatized or acetylated as appropriate, with traceable  $\delta^{13}\text{C}$  values.

## **2.2 Identification of urinary metabolites prior to reporting an Adverse Analytical Finding**

- It is specified that the GC-MS analysis to ensure the identity of the peaks of interest and the absence of interferences is also required prior to reporting an Atypical Finding.
- In addition, it is established that a full scan spectrum shall be obtained over the complete width of the steroid chromatographic peak(s) of interest to document the lack of interference.

## **2.3 Interpretation of GC-C-IRMS results**

- In this section, the criteria for interpreting the results of GC-C-IRMS analyses as "positive", "inconclusive" or "negative" are further clarified.
- In order to achieve further harmonization of laboratory results reporting, the requirement to consider the "laboratory reference ranges" (*i.e.* that the  $\Delta\delta$  value of the diagnostic ERC-TC pair in the sample shall be greater than the laboratory's reference mean  $\Delta\delta + 3\text{SD}$  value) before concluding a GC-C-IRMS result as positive has been reviewed. Thus, criteria have been established for the reference measurements in section 2.1 GC-C-IRMS Method Characteristics, which the laboratory shall meet to conclude that the method is being applied correctly and is producing reliable results.

## **3.0 Reporting GC-C-IRMS Results**

- It has been emphasized that if a laboratory obtains a second opinion for a GC-C-IRMS result that indicates an Adverse Analytical Finding, the second opinion shall be included in the Laboratory Documentation Package.
- The laboratory shall also report the  $\delta^{13}\text{C}$  value of all TCs and ERC analyzed, and each associated  $u_c$ , for all atypical and negative GC-C-IRMS findings.