WORLD ANTI-DOPING AGENCY
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WADA Technical Letter – TL20

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Written by:	*WADA* LabEG	Approved by:	*WADA* Executive Committee
Date:	23 September 2019	Effective Date:	23 September 2019

IN SITU FORMATION OF SPECIFIC SUBSTANCES WITH A STEROID STRUCTURE

The *World Anti-Doping Agency* wishes to draw the attention of the Laboratories to the following issue that may affect Laboratory operations. This pertains, in particular, to the possible detection of urinary steroids resulting from the *in situ* transformation of endogenous steroids.

Some Anabolic Androgenic Steroids (AAS) and/or Hormone and Metabolic Modulators may be formed at low concentrations in *Samples* by *in situ* microbial transformation of endogenous steroids.[1] Therefore, Laboratories shall exercise caution before reporting a result as an *Adverse Analytical Finding* (*AAF*) for the following *Prohibited Substances*:

S.1a: **Exogenous AAS**

- 1-androstenediol (5α-androst-1-ene-3β,17β-diol);
- 1-androstenedione (5α-androst-1-ene-3,17-dione);
- 1-testosterone (17β-hydroxy-5α-androst-1-en-3-one); and
- 1-androsterone (3α-hydroxy-5α-androst-1-ene-17-one).

S.4: **Hormone and Metabolic Modulators**

- 2-Androstenol (5α-androst-2-en-17-ol);
- 2-Androstenone (5α-androst-2-en-17-one);
- 3-Androstenol (5α-androst-3-en-17-ol);
- 3-Androstenone (5α-androst-3-en-17-one);
- Androsta-1,4,6-triene-3,17-dione (androstatrienedione); and
- Androsta-3,5-diene-7,17-dione (arimistane).

Laboratories should consider the following course of action when detecting the presence of the above-mentioned *Prohibited Substances* at low concentrations - below the Minimum Required Performance Level (MRPL) [2]:

1. Perform a Confirmation Procedure (CP) using an extraction step (*e.g.*, Solid Phase Extraction (SPE)) prior to the enzymatic hydrolysis to avoid inducing the *in situ* formation of the target compound(s) through the enzymatic activity of microbes already present in the *Sample* [However, if the *in situ* formation of these steroids has already occurred prior to the enzymatic hydrolysis, SPE will have no impact].

2. Evaluate the overall pattern of *Metabolites* present in the *Sample* by following recent scientific literature.

3. Analysis by GC/C/IRMS is strongly recommended, when applicable, including the transfer of the relevant *Sample*(s) to another Laboratory if necessary (depending on Laboratory's analytical capacity).

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4. It is recommended that the Laboratory seeks a second opinion, in writing, from another Laboratory before reporting the *AAF*. The second opinion shall be recorded in the Laboratory Documentation Package.

Should you have any further questions, please do not hesitate to contact the *WADA* Science Department.

REFERENCES

1. Grosse J. *et. al.* Degradation of doping-relevant Steroids by Rh. Erythropolis. In Recent Advances in Doping Analysis (15), Schanzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Koln, 2007; 385.

2. *WADA* Technical Document TD MRPL: Minimum Required Performance Levels for Detection and Identification of Non-Threshold Substances. https://www.wada-ama.org/en/what-we-do/science-medical/laboratories.