

Document Number:	TL20	Version Number:	2.0
Written by:	WADA Science	Approved by:	WADA Executive Committee
Reviewed by:	WADA <u>Laboratory Expert Group</u>		
Date:	21 December 2020	Effective Date:	1 January 2021

***IN SITU* FORMATION OF SPECIFIC SUBSTANCES WITH A STEROID STRUCTURE**

1.0 Introduction

WADA wishes to draw the attention of the Laboratories to the possible detection of some **Anabolic Androgenic Steroids (AAS)** and/or **Hormone** and **Aromatase Inhibitors** in urine *Samples* resulting from the *in situ* microbial transformation of endogenous steroids ^[1].

2.0 Analysis and Reporting Requirements

Laboratories shall exercise caution before reporting a result as an *Adverse Analytical Finding (AAF)* for the following *Prohibited Substances*:

S1.1: AAS

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

S4.1: Aromatase Inhibitors

- 2-Androstenol (5 α -androst-2-en-17-ol);
- 2-Androstenone (5 α -androst-2-en-17-one);
- 3-Androstenol (5 α -androst-3-en-17-ol);
- 3-Androstenone (5 α -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 corresponding 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 Analyte(s) through the enzymatic activity of microbes already present in the *Sample*.

WADA Technical Letter – TL20

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[Comment: 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 (e.g. presence of phase-II *Metabolites*);
3. 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.

3.0 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: Köln, 2007; 385.
- [2] WADA *Technical Document* TD MRPL: Minimum Required Performance Levels for Detection and Identification of Non-Threshold Substances by Chromatographic-Mass Spectrometric Analytical Methods.

[Current versions of WADA Technical Documents may be found at <https://www.wada-ama.org/en/what-we-do/science-medical/laboratories>]