WADA Technical Letter – TL19

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Written by: Reviewed by:	*WADA* Science *WADA* Laboratory Expert Group	Approved by:	*WADA* Executive Committee
Date:	21 December 2020	Effective Date:	1 January 2021

IN SITU FORMATION OF PREDNISONE AND PREDNISOLONE

1.0 Introduction

WADA wishes to draw the attention of the Laboratories to the possible detection of **Prednisone** and/or **Prednisolone** in urine *Samples* resulting from *in situ* microbial transformation of **Cortisone** and **Cortisol**, respectively.

The endogenous glucocorticoids cortisol (also known as hydrocortisone) and cortisone have a 3-oxo-4-ene structure (Figure 1). This structural element is a possible microbial Δ^1-steroid-dehydrogenase (Δ^1-SDH) target for 1,2-dehydrogenation, which may transform cortisol and cortisone into prednisolone and prednisone, respectively [1].

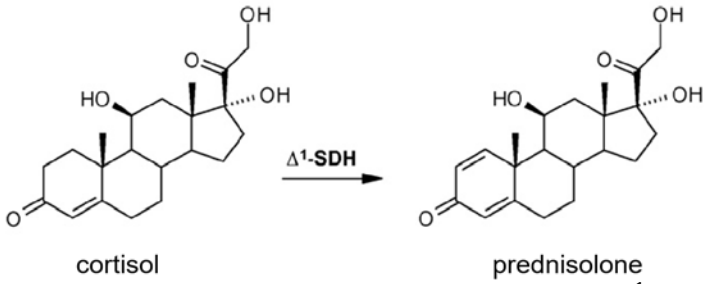

Figure 1. The microbial conversion of cortisol to prednisolone by Δ^1-SDH enzymatic activity.

2.0 Analysis and Reporting Requirements

Before reporting a result as an *Adverse Analytical Finding* (*AAF*) for prednisone and/or prednisolone, Laboratories shall evaluate whether the finding is the result of the *in situ* microbial transformation of cortisone and cortisol, respectively.

1. When prednisone and/or prednisolone are present at levels higher than (>) the *Minimum Reporting Limit* (*MRL*) of 30 ng/mL, perform a CP using an extraction method [*e.g.,* Solid Phase Extraction (SPE)] prior to the enzymatic hydrolysis step in order to avoid inducing the *in situ* formation of prednisone and prednisolone by the enzymatic activity of microbes already present in the *Sample.*

   [Comment: However, if the in situ formation of prednisone and prednisolone have already occurred prior to the enzymatic hydrolysis, SPE will have no impact.]

2. Evaluate the overall pattern of the *Metabolites* present in the *Sample*: the presence of aglycones only (*i.e.*, absence of the glucuronide *Metabolites*) and the absence of the 20β-hydroxy-*Metabolite* can be used as an indication of microbial activity.

3. Perform GC/C/IRMS [2] analysis (depending on Laboratory's analytical capacity, which may require the subcontracting of the analysis) when prednisone and/or prednisolone are present at levels (after correction for SG, if needed) estimated between (≥) 30 ng/mL and (≤) 60 ng/mL.

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4. Report the result as a Negative Finding if:

   – Prednisone and/or prednisolone are detected at an estimated concentration (SG-adjusted, if needed), which is lower than (<) the *MRL* of 30 ng/mL; or

   – The estimated concentration of prednisone and/or prednisolone (SG-adjusted, if needed) is between (≥) the *MRL* of 30 ng/mL and (≤) 60 ng/mL, but:

   - The overall pattern of relevant substances and *Metabolites* present in the *Sample* indicates microbial activity (*e.g.*, absence of 20β-hydroxy- and glucuronide-*Metabolites* and/or indication of a Δ^1-SDH activity); and/or
   - The GC/C/IRMS analysis demonstrates an endogenous origin of the substance(s) [2].

5. Report the result as an *Atypical Finding* (*ATF*) if:

   – The estimated concentration of prednisone and/or prednisolone (SG-adjusted, if needed) is between (≥) 30 ng/mL and (≤) 60 ng/mL, and the GC/C/IRMS analysis is inconclusive or cannot be performed [2];

   – The estimated concentration (SG-adjusted, if needed) of prednisone and/or prednisolone in the *Sample* is higher than (>) 60 ng/mL, but the *Sample* shows signs of extensive degradation;

   – If an *ATF* is reported, the Laboratory shall include a comment in the *ADAMS* Test Report recommending the *Testing* Authority to conduct at least one (1) follow-up no-notice test on the *Athlete* within a reasonable time frame (*e.g.* within 2 weeks).

6. Report the result as an *Adverse Analytical Finding* (*AAF*) if:

   – The confirmed estimated concentration (SG-adjusted, if needed) of prednisone and/or prednisolone in the *Sample* is higher than (>) 60 ng/mL, and there are no signs of extensive degradation; or

   – The estimated concentration of prednisone and/or prednisolone (SG-adjusted, if needed) is between (≥) 30 ng/mL and (≤) 60 ng/mL, and the GC/C/IRMS analysis demonstrates an exogenous origin of the substance(s) [2];

   – 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] Bredehöft M., *et. al.* Investigations of the microbial transformation of cortisol to prednisolone in urine samples. *J Steroid Biochem Mol Biol.* **129**(1-2): 54-60, 2012.

[2] *WADA Technical Document* TD IRMS: Detection of Synthetic Forms of *Prohibited Substances* by GC/C/IRMS.

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