WADA Technical Letter – TL19

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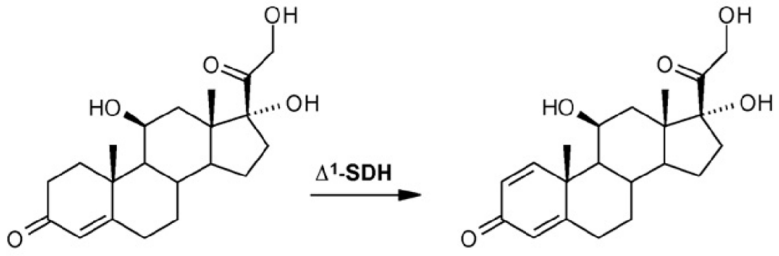
Written by:	*WADA* LabEG	Approved by:	*WADA* Executive Committee
Date:	23 September 2019	Effective Date:	23 September 2019

IN SITU FORMATION OF PREDNISONE AND PREDNISOLONE

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 prednisone and/or prednisolone in urine *Samples* resulting from *in situ* microbial transformation of cortisone and cortisol, respectively.

This Technical Letter constitutes supplementary information to the Technical Letter TL09 "*In situ* Formation of Exogenous Compounds in urine *Samples*" (previously, TL03/2017).

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]



cortisol prednisolone

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

Therefore, when reporting findings for prednisone and/or prednisolone, Laboratories should consider the following recommendations:

1. When prednisone and/or prednisolone are present at levels higher than (>) the reporting limit of 30 ng/mL, perform a Confirmation Procedure 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* [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 analysis (following consultation with the Testing Authority and 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 [2]) higher than (>) the reporting limit of 30 ng/mL and lower than (<) 60 ng/mL.

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Reporting of Results

- The finding shall be reported as a Negative Finding if:

  o Prednisone and/or prednisolone are detected at an estimated concentration (SG-adjusted, if needed [2]), which is lower than (<) the reporting limit of 30 ng/mL; or

  o The estimated concentration of prednisone and/or prednisolone (SG-adjusted, if needed [2]) is higher than (>) the reporting limit of 30 ng/mL and lower than (<) 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).

- The finding shall be reported as an *Atypical Finding* if:

  o The estimated concentration of prednisone and/or prednisolone (SG-adjusted, if needed [2]) is higher than (>) the reporting limit of 30 ng/mL and lower than (<) 60 ng/mL, and the GC/C/IRMS analysis is inconclusive or cannot be performed.

  o 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).

- The finding shall be reported as an *Adverse Analytical Finding* if:

  o The estimated concentration (SG-adjusted, if needed [2]) of prednisone and/or prednisolone in the *Sample* is higher than (>) 60 ng/mL and the evaluated metabolic pattern does not indicate microbial activity; or

  o The estimated concentration of prednisone and/or prednisolone (SG-adjusted, if needed [2]) is higher than (>) the reporting limit of 30 ng/mL and lower than (<) 60 ng/mL, and the GC/C/IRMS analysis demonstrates an exogenous origin of the substance(s).

  o 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.

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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 Endogenous Anabolic Androgenic Steroids by GC/C/IRMS. https://www.wada-ama.org/en/what-we-do/science-medical/laboratories