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 Δ₁-steroid-dehydrogenase (Δ₁-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 Δ₁-SDH enzymatic activity.](image)

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.
Reporting of Results

- The finding shall be reported as a **Negative Finding** if:
  - Prednisone and/or prednisolone are detected at an estimated concentration (SG-adjusted, if needed) which is lower than (<) the reporting limit of 30 ng/mL; or
  - The estimated concentration of prednisone and/or prednisolone (SG-adjusted, if needed) 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 Δ1-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:
  - The estimated concentration of prednisone and/or prednisolone (SG-adjusted, if needed) 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.
  - 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:
  - The estimated concentration (SG-adjusted, if needed) 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
  - The estimated concentration of prednisone and/or prednisolone (SG-adjusted, if needed) 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).
  - 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
