

WADA Technical Document – ISL TD2027NA

Summary of Major Modifications

This document summarizes the major modifications in the TD2027NA. In addition, a redline version against the TD2021NA v2.0, which is the current version in force, is also presented for reference.

Key updates and modifications include:

- 1. Effective Date:** The effective date is 01 January 2027.
- 2. Title:** The term “related to Nandrolone” has been removed, since this TD focuses on the analysis and reporting of other 19-norsteroids in addition to nandrolone.
- 3. 19-NA from the consumption of edible parts of non-castrated male pigs or cryptorchids:** It has been clarified that these rare cases shall not be considered an endogenous source of 19-NA, since the substance originates from the consumed food.
- 4. Analysis of 19-NE:** The revised TD clarifies that in some cases, the analysis of the minor metabolite 19-NE may be needed to establish the Use of 19-norsteroids (see Article 3.2.1.2.1)
- 5. Article 2.0 – Procedure for the Analysis of 19-Norsteroids:** This Article clarifies that for the estimation of 19-NA and 19-NE concentrations by GC-MSⁿ, the Laboratory shall target, both during the Initial Testing Procedure (ITP) and the Confirmation Procedure (CP), the total content of free steroid obtained from the non-conjugated steroid plus the free steroid fraction released after hydrolysis of the respective phase-II glucuronidated metabolite.
- 6. Article 3.1 – Initial Testing Procedure:** This Article includes ITP validation requirements according to the new ISL TD2027VAL, a Limit of Detection (LOD) not higher than 1.25 ng/mL and monitoring the efficiency of hydrolysis and derivatization in each test Aliquot. In addition, method performance characteristics are defined such as the use of a Single Point Calibrator (SPC) as well as negative and positive quality control (QC) samples. In addition, it has been clarified that the 19-NA (or 19-NE) concentration estimated during the ITP shall not be corrected for the specific gravity (SG) to subject a sample to the CP.
- 7. Article 3.2.1.1 – “A” Sample GC-MSⁿ Confirmation Procedure:** This Article now aligns the CP for 19-norsteroids to MRL-based confirmation approach for non-threshold substances subject to MRL (new TD ISL2027MRL): the use of a PQC at MRL (15 ng/mL) and an SPC at 150% MRL (22.5 ng/mL), prepared from different batches or different stock solutions of Reference Material, to determine whether the estimated concentration in the sample exceeds the MRL with sufficient statistical confidence.
- 8. Article 3.2.1.2 – “A” Sample GC/C/IRMS Analysis:** A structured decision tree for the conduct of GC/C/IRMS analysis is provided table 1, including clarification on when GC/C/IRMS analysis is not needed/required/optional, with explanatory footnotes clarifying the different scenarios as well as the adjustment of the estimated concentration when the SG > 1.018 before deciding whether the GC/C/IRMS analysis is required.
- 9. Article 3.2.1.2.2 – GC/C/IRMS CP Validation and Analysis Requirements:** The 19-NA/19-NE GC/C/IRMS validation and analysis requirements have been removed since they will be included in the revised ISL TD2027IRMS,

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- 10. Article 3.2.1.2.3 – Interpretation of GC/C/IRMS Results:** Better instructions are given for the investigation of 19-NA cases where the Athlete invokes the consumption of edible parts of intact pigs or cryptorchids as the unlikely origin of the 19-NA finding.
 - 11. Article 3.2.1.3.2 – Test for Demethylation:** The Article now clarifies that 19-NE may also be produced in urine samples, in small concentrations, by *in situ* 19-demethylation of Etiocholanolone (Etio), and that this can be verified by GC/C/IRMS analysis.
 - 12. Article 3.2.2 – “B” Sample Confirmation Procedure:** The Article now clarifies that when the AAF for the “A” sample is based on the GC/C/IRMS results, the “B” sample CP requires only the GC/C/IRMS analysis.
 - 13. Article 4.0 – Interpretation and Reporting of Results:** Instructions are given to refer to the ISL TD2027IRMS for the reporting of GC/C/IRMS results. In addition, table 2 has been updated to better reflect the different test results and reporting scenarios described in the document.
 - 14. Annex A – Confirmation Procedure for 19-Nosteroids:** The follow chart has been updated according to the changes made to the TD, including the separation of the CP strategy t be followed for 19-NA and 19-NE determinations.