# *WADA Technical Document* – ISL TD2027NA

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| Written by:<br>Reviewed by: | *WADA* Science/NA Working Group<br>*WADA* Laboratory Expert Group | Approved by: | *WADA* Executive Committee |
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## HARMONIZATION OF ANALYSIS AND REPORTING OF 19-NORSTEROIDS

### 1.0 Introduction

This *Technical Document* (*TD*), which constitutes an integral part of the *International Standard* for Laboratories (ISL [1]), has been established to harmonize the analysis of 19-norsteroids in urine and the reporting of 19-norsteroids findings by Laboratories.

The detection of the *Use* of 19-norsteroids (*e.g.,* 19-nortestosterone (nandrolone), 19-norandrostenedione, 19-norandrostenediol) is based primarily upon the identification of the main urinary *Metabolite*, 19-norandrosterone (19-NA). More than one *Metabolite* of administered 19-norsteroids may be detected in urine *Samples* and reported [*e.g.,* 19-noretiocholanolone (19-NE)]; however, the identification of 19-NA, including the demonstration, where required, that the 19-NA is not of endogenous origin, is generally sufficient to report an *Adverse Analytical Finding (AAF*). In some cases, the analysis of 19-NE may be needed to establish the *Use* of 19-norsteroids (see Article 3.2.1.2.1)

*[Comment to Article 1.0: In the context of this ISL TD, "endogenous" origins of 19-NA at levels higher than 2.5 ng/mL include: i) pregnancy [2-4], and ii) in situ microbial degradation of androsterone (A) to 19-NA [5]. This ISL TD provides elements to determine the source of 19-NA in each of these cases. In rare cases, the consumption of edible parts of non-castrated male pigs or cryptorchids can also result in 19-NA at levels higher than 2.5 ng/mL [4, 6-12].]*

### 2.0 Procedure for the Analysis of 19-Norsteroids

The analysis of 19-norsteroids follows a two (2)-step procedure:

a) An Initial *Testing* Procedure (ITP) (see Article 3.1) based on the Gas Chromatography-Mass Spectrometric (GC-MS$^n$) detection and estimation of the concentration of 19-NA (and 19-NE, where necessary – see also Article 3.2.1.2.1), and

b) A subsequent Confirmation Procedure (CP) (see Article 3.2), which consists of the GC-MS$^n$ identification (as per ISL *TD* IDCR [13]) and estimation of the concentration of 19-NA and/or 19-NE (where necessary), and the eventual performance of Gas Chromatography/Combustion/Isotope Ratio Mass Spectrometry (GC/C/IRMS) analysis (see Article 3.2.1.2).

For the estimation of 19-NA and 19-NE concentrations by GC-MS$^n$, the Laboratory shall target, both during the ITP and the 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* with an enzyme with substrate specificity for β-D-glucuronide linkages (*e.g.*, purified β-glucuronidase from *E. coli* or other Fit-for-Purpose enzyme with the same substrate specificity, as determined by the Laboratory during Test Method validation).

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## 3.0 Analytical *Testing* Procedure Requirements

### 3.1 Initial *Testing* Procedure

The ITP of 19-norsteroids includes the GC-$MS^n$ detection and estimation of the concentration of 19-NA (and 19-NE, where necessary).

a) The ITP shall be validated according to the ITP validation requirements established in the ISL *TD* VAL [14].

- The Limit of Detection (LOD) of the ITP for 19-NA and 19-NE, as estimated during Test Method validation, shall be less than or equal to (≤) 1.25 ng/mL.

b) The ITP shall include the enzymatic hydrolysis of the 19-NA (and 19-NE, where necessary) glucuronide using an enzyme with substrate specificity for β-D-glucuronide linkages (*e.g.*, purified β-glucuronidase from *E. coli*). The efficiency of hydrolysis shall be monitored in each test Aliquot with isotopically labeled Androsterone (A)-glucuronide (or an equivalent scientifically recognized steroid glucuronide alternative).

c) The Aliquot shall be subject to derivatization to produce TMS derivatives of the target *Metabolite*(s) (TMS enol ethers and/or TMS ethers). The efficiency of the derivatization shall be controlled in each test Aliquot through the monitoring of mono-O-TMS vs. di-O-TMS derivative of A.

d) The ITP shall include the following characteristics:

i. A Single Point Calibrator (SPC) prepared in urine at a concentration of 19-NA (or 19-NE, where necessary) between 2.5-15 ng/mL.

*[Comment to Article 3.1 d): Alternatively, at the Laboratory's discretion and according to its Test Method validation results, a calibration curve in urine with a working range of at least 2.5-15 ng/mL may also be used.]*

ii. A urine NQC with an estimated concentration of 19-NA (or 19-NE, where necessary), if detected in the sample, not higher than (≤) 1.25 ng/mL.

iii. A urine PQC containing 19-NA (or 19-NE, where necessary) at a concentration higher than (>) 2.5 ng/mL. It is recommended that the PQC be prepared at a different concentration than the SPC.

iv. An appropriate labeled (*e.g.,* $^2H$- or $^{13}C$-) steroid internal standard (ISTD).

e) 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,

## 3.2 Confirmation Procedure

### 3.2.1 "A" *Sample* Confirmation Procedure

The "A" CP of 19-norsteroids includes the GC-MS$^n$ estimation of the concentration and identification (in compliance with the ISL *TD* IDCR [13]), as well as the eventual GC/C/IRMS analysis, of 19-NA (or 19-NE, where necessary) to establish the origin (endogenous or exogenous) of the *Metabolite*(s) detected.

#### 3.2.1.1 "A" *Sample* GC-MS$^n$ Confirmation Procedure

The GC-MS$^n$ CP for 19-norsteroids shall be validated according to the CP Qualitative Procedure validation requirements established in the ISL *TD* VAL [14].

a) The Limit of Identification (LOI) of the GC-MS$^n$ CP for 19-NA and 19-NE, as estimated during Test Method validation, shall be less than (<) 2.5 ng/mL.

b) Like the ITP, the GC-MS$^n$ CP shall include the enzymatic hydrolysis of the glucuronidated *Metabolite*(s) and the derivatization to produce TMS derivatives (TMS enol ethers and/or TMS ethers). The efficiency of both processes shall be monitored in each CP Aliquot (see Articles 3.1 b) and c).

c) The GC-MS$^n$ CP for 19-NA shall follow the requirements for the confirmation of Non-Threshold Substances subject to a *Minimum Reporting Level* (*MRL*), as specified in the ISL *TD MRL* [15], including the use of:

   i. A PQC urine sample at the *MRL* of 15 ng/mL.

   ii. A Single Point Calibrator (SPC) [1] prepared in urine at 150% *MRL* (22.5 ng/mL).

   iii. A urine NQC with an estimated concentration of 19-NA (or 19-NE, where necessary), if detected in the sample, not higher than (≤) 1.25 ng/mL.

   iv. An appropriate labeled ISTD (*e.g.,* $^2$H-19-NA glucuronide).

*[Comment 1 to Article 3.2.1.1: No quantification (and, therefore, no Measurement Uncertainty estimation) is required in the GC-MS$^n$ CP. The use of the SPC at 22.5 ng/mL and appropriate QC samples is sufficient to confirm the estimated concentration of 19-NA (or 19-NE, where necessary.]*

*[Comment 2 to Article 3.2.1.1: The SPC, NQC and PQC shall be subjected to the same sample preparation procedure as the Sample Aliquot.]*

[1] The SPC (or calibration curve) shall be prepared from a different batch or different stock solution of Reference Material than the PQC. For the SPC, it is preferably that the concentration is based on/traceable to a Certified Reference Material.

3.2.1.2 "A" Sample GC/C/IRMS Analysis

3.2.1.2.1 Conducting GC/C/IRMS Analysis

a) The conditions for performing GC/C/IRMS analysis for 19-NA are described in Table 1 below.

b) The Laboratory should consider conducting GC/C/IRMS analysis for 19-NE when the GC/C/IRMS analysis of 19-NA is not conclusive (*e.g.*, presence of interferences or when 5α-reductase inhibitor activity is confirmed in the *Sample*).

c) If the Laboratory does not have the analytical capacity to perform GC/C/IRMS analysis for 19-NA (or 19-NE, if applicable), the Laboratory shall, in consultation with the *Testing* Authority (TA) (or the *Results Management* Authority (RMA), if different), have *Samples* transferred to another Laboratory that has such analytical capacity (Analytical Method included in the Laboratory's Scope of ISO/IEC 17025 Accreditation).

**Table 1** GC/C/IRMS analysis of 19-NA

| GC/C/IRMS analysis | Estimated concentration | ***Samples*** |
|---|---|---|
| Not needed | • > 15 ng/mL [2, 3]<br>**(*AAF*)** | • Males<br>• Females<br>– Non-pregnant, and<br>– Absence of 3,5-tetrahydronorethisterone (THNE) |
| | • ≤ 15 ng/mL [2, 3] | • Females<br>– Presence of THNE |
| **Required** | • 2.5 - 15 ng/mL [2, 4] | • Males<br>• Females<br>– Non-pregnant, and<br>– Absence of THNE |
| | • > 15 ng/mL [2, 3] | • Females<br>– Pregnant females |
| Optional | • 2.5 - 15 ng/mL [4] | • Females<br>– Pregnant females |
| | • < 2.5 ng/mL | • Males<br>• Females<br>– Non-pregnant<br>– Absence of THNE |

[2] According to the ISL *TD MRL* [15], only when the estimated concentration of 19-NA (or 19-NE, where applicable) in the *Sample* exceeds that of the 150% *MRL* SPC, and the estimated concentration of 19-NA (or 19-NE, where applicable) in the 100% *MRL* PQC is lower than (<) the respective concentration in the SPC, the Laboratory can conclude with enough confidence that the concentration of 19-NA in the *Sample* is higher than (>) the *MRL* of 15 ng/mL.

For the avoidance of doubt, when the analysis does not fulfill these requirements, *e.g.*, when the estimated concentration of 19-NA is higher than (>) the 100% *MRL* PQC but lower than (<) the 150% *MRL* SPC, the Laboratory cannot conclude with sufficient confidence that the concentration of 19-NA in the *Sample* is higher than (>) the *MRL*. Therefore, in those cases, the Laboratory shall subject the *Sample* to GC/C/IRMS analysis (or subcontract the analysis to another Laboratory with the required analytical capacity) to establish the origin of 19-NA in the *Sample* and determine whether the result constitutes an *AAF* or not.

[3] When the SG of the *Sample* ($SG_{Sample}$), as measured by the Laboratory during the CP, is higher than (>) 1.018, and the GC-$MS^n$ CP-estimated concentration of 19-NA in the *Sample* is confidently greater than (>) the *MRL* of 15 ng/mL (as determined in accordance with the ISL *TD MRL*) [15], the estimated 19-NA concentration shall be adjusted for the SG of 1.020 [15]. Only when the SG-adjusted concentration is > 15 ng/mL the result can be reported as an *AAF* without the need for GC/C/IRMS analysis.

[4] In such cases, the adjustment of the 19-NA concentration for a high $SG_{Sample}$ (> 1.018) is not necessary, since it would only lead to a lower adjusted concentration, whereas the actual concentration in the *Sample*, without the adjustment for the $SG_{Sample}$, should allow the performance of the GC/C/IRMS analysis

3.2.1.2.2 GC/C/IRMS CP Validation and Analysis Requirements

Refer to the ISL *TD* IRMS [16] for general GC/C/IRMS Test Method validation and analysis requirements.

3.2.1.2.3 Interpretation of GC/C/IRMS Results

a) To reject the hypothesis of endogenous or *in situ* 19-NA (or 19-NE, where applicable) formation based on the application of GC/C/IRMS analysis (*i.e.,* to report the finding as an *AAF*), the $|\Delta\delta^{13}C|$ values between two (2) ERCs and 19-NA (or 19-NE, where applicable), *i.e.,* $|\Delta\delta^{13}C| = |\delta^{13}C_{ERC} - \delta^{13}C_{19\text{-NA (or 19-NE)}}|$, shall be greater than (>) 3.0 ‰ (refer also to the ISL *TD* IRMS [16] for use of ERCs and interpretation of GC/C/IRMS test results) .

*[Comment to Article 3.2.1.2.4 a): Androsterone (A) shall not be used as an ERC for 19-NA GC/C/IRMS analysis when there are indications of the administration of testosterone or its precursors (e.g., prasterone). In such cases, an alternative ERC as described in ISL TD IRMS [16] shall be used.]*

b) Due to the occurrence of preparations of 19-norsteroids with a carbon isotopic signature ($^{13}C/^{12}C$) close to that of endogenous human urinary steroids (*e.g.*, $\delta^{13}C_{19\text{-NA}}$ between -16.000 ‰ and -24.000 ‰) [11, 17], the result of the GC/C/IRMS analysis of the excreted 19-NA (or 19-NE) may not always readily indicate its exogenous origin in *Samples* (result of the GC/C/IRMS analysis negative or inconclusive).

c) If the consumption of edible parts of intact pigs or cryptorchids is invoked by an *Athlete* as the unlikely origin of a 19-NA finding, the TA (or RMA, if different) shall consider the compatibility of any previously collected and/or follow-up *Samples* (viz. the analytical results) with that explanation in terms of 19-NA excretion pattern in

urine. The excretion kinetics of 19-NA and 19-NE following oral ingestion will have a different time course than after an injection of 19-norsteroids [6, 9, 12, 19-21].

3.2.1.3 Additional Tests

3.2.1.3.1 Tests for Norethisterone and Pregnancy

19-NA is excreted during pregnancy [2-4] and as a minor *Metabolite* of norethisterone [18, 22, 23], a progestogen agent that is present in some oral contraceptives of permitted use in some countries. Therefore, when the estimated concentration of 19-NA is equal to or exceeds (≥) 2.5 ng/mL in the urine *Sample* of a female *Athlete,* the Laboratory shall:

a) Test for pregnancy based on the measurement of urinary human Chorionic Gonadotrophin (hCG) in the urine *Sample*.

b) Establish the presence or absence of 3,5-tetrahydronorethisterone (THNE), the main *Metabolite* of norethisterone, and whether it is compatible with the 19-NA concentration.

*[Comment to Article 3.2.1.3.1 b): When testing for the presence of THNE, Laboratories should target the 3$\alpha$,5$\beta$ isomer, namely 5$\beta$-estran-17$\alpha$-ethynyl-3$\alpha$,17$\beta$-diol [23]. With 19-NA being a minor Metabolite of norethisterone, 19-NA concentrations at levels higher than (>)15 ng/mL should be associated with an estimated concentration ratio of 19-NA / 5$\beta$-estran-17$\alpha$-ethynyl-3$\alpha$,17$\beta$-diol ≤ 2 in the Sample.]*

3.2.1.3.2 Test for Demethylation

In addition, 19-NA and 19-NE may be produced in urine *Samples*, in small concentrations, by *in situ* 19-demethylation of androsterone (A) and Etiocholanolone (Etio), respectively [5]. This potential *in situ* formation of 19-NA and 19-NE is verified by GC/C/IRMS analysis [24].

3.2.2 "B" *Sample* Confirmation Procedure

a) In cases 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.

b) In cases when the estimated concentration of 19-NA (SG-adjusted, if needed) is shown to be greater than (>) 15 ng/mL (in accordance with ISL *TD MRL* [15] requirements) in a *Sample* collected from a male or a non-pregnant female *Athlete*, the "B" *Sample* CP requires only the identification of 19-NA only, in accordance with the ISL *TD* IDCR [13].

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## 4.0 Interpretation and Reporting of Results

a) The Laboratory shall report 19-NA (and/or 19-NE, where applicable) findings in a *Sample* from a male or a female *Athlete* as defined in the Table below (see also flow diagram in Annex A).

b) Where GC/C/IRMS results are to be reported, the Laboratory shall refer to the ISL *TD* IRMS [16] for GC/C/IRMS results reporting requirements.

**Table 2** Interpretation and Reporting of Results for 19-Norsteroids

| ***Sample* Origin** | ***Adverse Analytical Finding*** | ***Atypical Finding*** | **Negative Finding** |
|---|---|---|---|
| **4.1 Pregnant Female *Athletes*** | **Results** | | |
| | Results of the GC/C/IRMS analysis are consistent with the exogenous origin of 19-NA. | – Estimated concentration of 19-NA > 15 ng/mL. [2, 3]<br>AND<br>– Results of GC/C/IRMS analysis are inconclusive or do not meet the criteria supporting an exogenous origin of 19-NA. | – Estimated concentration of 19-NA ≤ 15 ng/mL. [2, 3]<br>AND<br>– GC/C/IRMS analysis is either not performed, or the results are consistent with an endogenous origin of 19-NA. |
| | **Test Report**<br>(**No reference to the pregnancy status of the *Athlete* shall be reported)** | | |
| | – Estimated concentration of 19-NA, expressed as "≤15 ng/mL" or ">15 ng/mL" [2, 3].<br>– Results of GC/C/IRMS analysis, including a comment indicating that the GC/C/IRMS finding is consistent with an exogenous origin of 19-NA, the $\delta^{13}C$ values for 19-NA and the ERCs, as well as the respective $u_c$ (‰). | – Estimated concentration of 19-NA >15 ng/mL [2, 3].<br>– Results of GC/C/IRMS analysis, including a comment indicating that the GC/C/IRMS finding is inconclusive or does not meet the criteria supporting an exogenous origin of 19-NA, the $\delta^{13}C$ values for 19-NA and the ERCs as well as the respective $u_c$ (‰). | If GC/C/IRMS analysis is performed:<br>– Estimated concentration of 19-NA ≤ 15 ng/mL [2, 3].<br>– Include a comment indicating that the GC/C/IRMS finding is consistent with an endogenous origin of 19-NA, the $\delta^{13}C$ values for 19-NA and the ERC, as well as the respective $u_c$ (‰). |

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| ***Sample* Origin** | ***Adverse Analytical Finding*** | ***Atypical Finding*** | **Negative Finding** |
|---|---|---|---|
| **4.2 Female *Athletes* using Norethisterone** | **Results** | | |
| | – Estimated concentration of 19-NA > 15 ng/mL [2, 3].<br>– The estimated concentration ratio of 19-NA/THNE is > 2. | – Estimated concentration of 19-NA > 15 ng/mL [2, 3].<br>– The estimated concentration ratio of 19-NA/THNE is ≤ 2. | – Estimated concentration of 19-NA ≤ 15 ng/mL [2, 3] |
| | **Test Report** | | |
| | – Estimated concentration of 19-NA >15 ng/mL [2, 3].<br>– A comment describing the presence of THNE in the *Sample*.<br>*Example:*<br>*"19-NA was found in the Sample at an estimated concentration > 15 ng/mL. Tetrahydronorethisterone, a Metabolite of norethisterone, was also detected in the Sample. The estimated concentration ratio 19-NA/ THNE is incompatible with norethisterone being the exclusive source of 19-NA".* | – Estimated concentration of 19-NA >15 ng/mL [2, 3].<br>– A comment describing the presence of THNE in the *Sample*.<br>*Example:*<br>*"19-NA was found in the Sample at an estimated concentration > 15 ng/mL. Tetrahydronorethisterone, a Metabolite of norethisterone, was also detected in the Sample. The estimated concentration ratio 19-NA/ THNE is compatible with norethisterone being the exclusive source of 19-NA"* | **No reference to the use of norethisterone shall be reported.** |
| **4.3 Male or Female *Athletes* (neither pregnant nor using norethisterone)** | **Results** | | |
| | – Estimated concentration of 19-NA > 15 ng/mL. [2, 3]<br>OR<br>– Estimated concentration of 19-NA ≤ 15 ng/mL [2,3] and GC/C/IRMS results are consistent with an exogenous origin of 19-NA. | – Estimated concentration of 19-NA ≤ 15 ng/mL [2,3] and GC/C/IRMS results are inconclusive or do not meet the criteria supporting an exogenous origin of 19-NA.<br>OR | – Estimated concentration of 19-NA < 2.5 ng/mL (and too low to perform GC/C/IRMS analysis).<br>OR<br>– Estimated concentration of 19-NA between 2.5 - 15 ng/mL [2, 3] and GC/C/IRMS |

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| **4.3 Male or Female *Athletes* (neither pregnant nor using norethisterone)** (cont.) | OR<br>– 19-NA cannot be reliably analyzed in the *Sample* (*e.g.*, due to the presence of 5α-reductase inhibitor activity) and the GC/C/IRMS results for 19-NE are consistent with an exogenous origin of 19-NE. | – 19-NA cannot be reliably analyzed in the *Sample* (*e.g.*, due to the presence of 5α-reductase inhibitor activity) and the GC/C/IRMS results for 19-NE are inconclusive or do not meet the criteria supporting an exogenous origin of 19-NE. | results are consistent with an endogenous origin of 19-NA.<br>OR<br>– 19-NA cannot be reliably analyzed in the *Sample* (*e.g.*, due to the presence of 5α-reductase inhibitor activity) and the GC/C/IRMS results for 19-NE are consistent with an endogenous origin of 19-NE. |
| | **Test Report** | | |
| | – If 19-NA > 15 ng/mL with no GC/C/IRMS analysis:<br>• Estimated concentration of 19-NA >15 ng/mL [2, 3].<br>• For *Samples* from female *Athletes*, include a comment explaining that pregnancy and the use of norethisterone were excluded as the source of 19-NA.<br>*Example: "The 19-NA finding is not consistent with pregnancy or the use of norethisterone".*<br>– If 19-NA ≤ 15 ng/mL with positive GC/C/IRMS results<br>• Estimated concentration of 19-NA ≤ 15 ng/mL.<br>• Results of GC/C/IRMS analysis, including a comment indicating that the GC/C/IRMS finding is consistent with an exogenous origin of 19-NA, the $\delta^{13}C$ values for 19-NA | – If 19-NA ≤ 15 ng/mL with inconclusive GC/C/IRMS results:<br>• Estimated concentration of 19-NA ≤ 15 ng/mL [2, 3].<br>• Results of GC/C/IRMS analysis, including a comment indicating that the GC/C/IRMS results are inconclusive (*e.g.,* due to the presence of interfering compound(s) or any other factor preventing a reliable GC/C/IRMS analysis) or do not meet the criteria supporting an exogenous origin of 19-NA, the $\delta^{13}C$ values for 19-NA and the ERCs as well as the respective $u_c$ (‰).<br>• For *Samples* from female *Athletes*, clarify that pregnancy was excluded as the source of 19-NA | – If GC/C/IRMS analysis is performed:<br>• Estimated concentration of 19-NA (either < 2.5 ng/mL or between 2.5-15 ng/mL).<br>• Results of GC/C/IRMS analysis, including a comment indicating that the GC/C/IRMS analysis is consistent with an endogenous origin of 19-NA, the $\delta^{13}C$ values for 19-NA and ERC as well as the respective $u_c$ (‰).<br>– If the finding is based on negative GC/C/IRMS results for 19-NE:<br>• Results of GC/C/IRMS analysis, including a comment indicating that the GC/C/IRMS analysis is consistent with an endogenous origin of 19-NE, the $\delta^{13}C$ values for 19-NE and the ERC as |

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| **4.3 Male or Female *Athletes* (neither pregnant nor using norethisterone)** (cont.) | and the ERCs as well as the respective $u_c$ (‰).<br>• For *Samples* from female *Athletes*, a comment explaining that the use of norethisterone was excluded as the source of 19-NA (*e.g.,* "the 19-NA finding is not consistent with the use of norethisterone").<br>– If the finding is based on positive GC/C/IRMS results for 19-NE:<br>– Results of GC/C/IRMS analysis, including a comment indicating that the GC/C/IRMS finding is consistent with an exogenous origin of 19-NE, the $\delta^{13}C$ values for 19-NE and the ERCs as well as the respective $u_c$ (‰). | *Example: "The 19-NA finding is not consistent with pregnancy".*<br>• Recommend the TA (or RMA, if different) to conduct follow-up no-notice tests on the *Athlete* as soon as possible and evaluate the kinetics of 19-NA excretion in urine.<br>– If the finding is based on inconclusive GC/C/IRMS results for 19-NE:<br>– Results of GC/C/IRMS analysis, indicating that the GC/C/IRMS results are inconclusive (*e.g.,* due to the presence of interfering compound(s) or any other factor preventing a reliable GC/C/IRMS analysis) or do not meet the criteria supporting an exogenous origin of 19-NE, the $\delta^{13}C$ values for 19-NE and the ERCs as well as the respective $u_c$ (‰). | well as the respective $u_c$ (‰). |

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## 5.0 References

[1] World Anti-Doping *Code International Standard* for Laboratories (ISL).

[2] Reznik Y *et al*. Rising levels of 19-norstestosterone throughout pregnancy: determination by radioimmunoassay and validation by gas chromatography-mass spectrometry. *J Clin Endocrinol Metab* **63**:1086–108, 1987.

[3] Mareck-Engelke U *et al*. 19-norandrosterone in pregnant women. In: Schanzer W, Geyer H, Gotzman A, *et al*. *Recent Advances in Doping Analysis* (8), Proceedings of the 18th Cologne Workshop on Dope Analysis. Cologne: Sport & Buch Straub: 145–54, 2000.

[4] Guay C *et al*. Excretion of norsteroids' phase II metabolites of different origin in humans. *Steroids* **74**: 350-8, 2009.

[5] Grosse J *et al*. Formation of 19-norsteroids by *in situ* demethylations of endogenous steroids in stored urine samples. *Steroids* **70**: 499-506, 2005.

[6] Debruyckere G, de Sagher R, Van Peteghem C. Influence of the consumption of meat contaminated with anabolic steroids on doping tests. *Anal Chim Acta* **275**: 49–56, 1993.

[7] Le Bizec B *et al*. Consequence of boar edible tissue consumption on urinary profiles of nandrolone metabolites. I. Mass spectrometric detection and quantification of 19-norandrosterone and 19-noretiocholanolone in human urine. *Rapid Commun. Mass Spectrom*. **14**: 1058-65, 2000.

[8] De Wasch K *et al*. Consequence of boar edible tissue consumption on urinary profiles of nandrolone metabolites. II. Identification and quantification of 19-norsteroids responsible for 19-norandrosterone and 19-noretiocholanolone excretion in human urine. *Rapid Commun. Mass Spectrom*. **15**: 1442-47, 2001.

[9] Poelmans S *et al*. Endogenous occurrence of some anabolic steroids in swine matrices. *Food Additives and Contaminants* **22**(9): 808–815, 2005.

[10] Ayotte C. Significance of 19-norandrosterone in athletes' urine samples. *Br. J. Sports Med.* **40**: 25-29, 2006.

[11] Hülsemann F *et al*. Case Study: Atypical $\delta^{13}C$ values of urinary norandrosterone. *Drug Test Anal*. **10**(11-12):1728-1733, 2018.

[12] Hülsemann F *et al*. Excretion of 19-norandrosterone after consumption of boar meat. *Drug Test Anal*. **12**(11-12):1581-1586, 2020.

[13] *WADA Technical Document* ISL *TD* IDCR: Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for *Doping Control* Purposes.

[14] *WADA Technical Document* ISL *TD* VAL: Minimum Requirements for Validation of Analytical *Testing* Procedures for *Doping Control.*

[15] *WADA Technical Document* ISL *TD MRL*: *Minimum Reporting Levels* applied in *Doping Control*.

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

[17] Brailsford AD *et al*. IRMS delta values (13 C) of nandrolone and testosterone products available in the UK: Implications for anti-doping. *Drug Test Anal*. **10**(11-12):1722-1727, 2018

[18] Walker CJ *et al*. Doping in sport - 1. Excretion of 19-norandrosterone by healthy women, including those using contraceptives containing norethisterone. *Steroids* **74**: 329-35, 2009.

[19] Bagchus WM *et al*. Pharmacokinetic evaluation of three different intramuscular doses of nandrolone decanoate: analysis of serum and urine samples in healthy men. *J Clin Endocrtinol Metab* **90**(5): 2424-30, 2005.

[20] Watson P *et al*. Urinary nandrolone metabolite detection after ingestion of a nandrolone precursor. *Med Sci Sports Exerc*. **41**(4):766-72, 2009.

[21] Palonek E *et al*. Atypical excretion profile and GC/C/IRMS findings may last for nine months after a single dose of nandrolone decanoate. *Steroids* **108**: 105-11, 2016.

[22] Van Eenoo P *et al*. In: W Schänzer, H Geyer, A Gotzmann, U Mareck (eds.) Recent Advances In Doping Analysis (14). Sport und Buch Strauß - Köln 2006; pp. 189-197

[23] Walker CJ *et al*. Doping in sport: 3. Metabolic conversion of oral norethisterone to urinary 19-norandrosterone. *Steroids* **74**:341-349, 2009.

[24] Hebestreit M *et al*. Determination of the origin of urinary norandrosterone traces by gas chromatography combustion isotope ratio mass spectrometry. *Analyst* **131**:1021-6, 2006.

*[Current versions of WADA's ISL and ISL TDs may be found at https://www.wada-ama.org/en/what-we-do/international-standards]*

# *WADA Technical Document* – ISL TD2027NA

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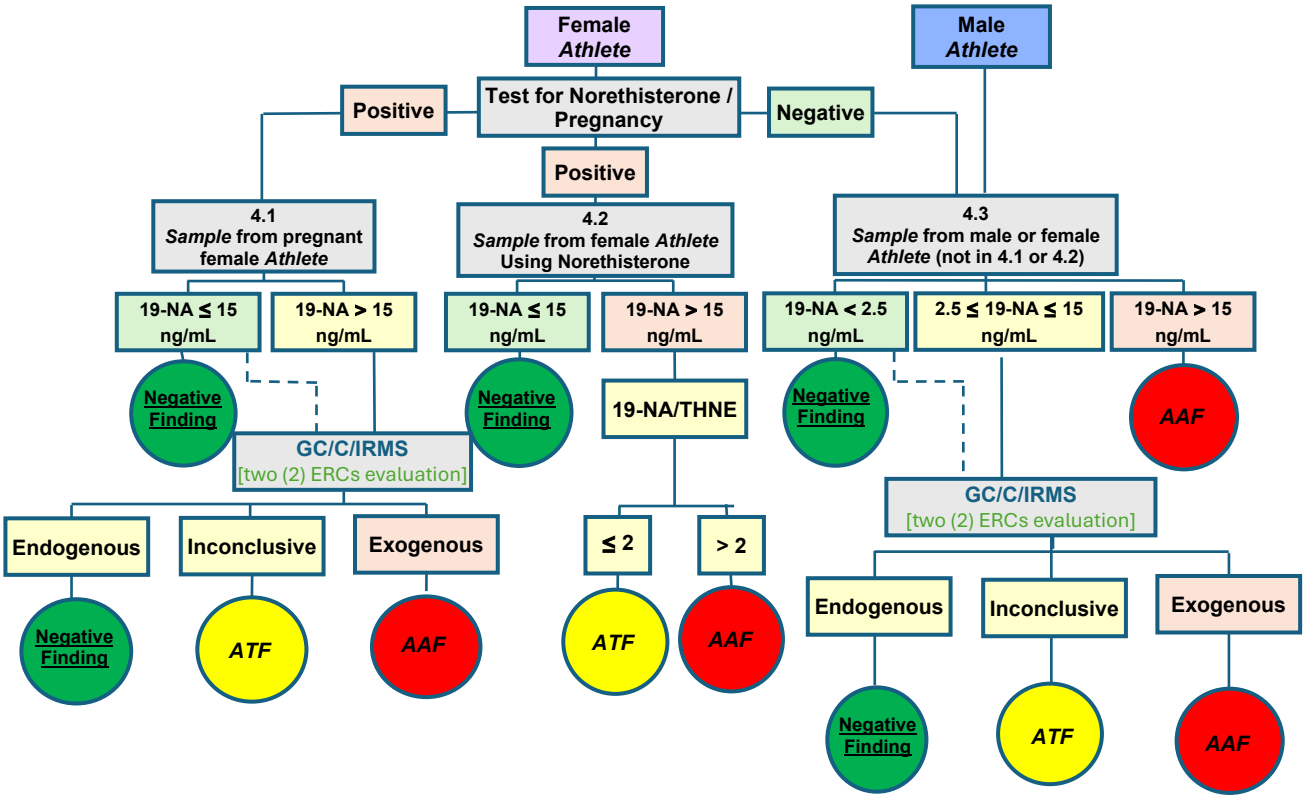
## ANNEX A. Confirmation Procedure for 19-Norsteroids

### A1. Confirmation Procedure for 19-NA




### A.2 Confirmation Procedure for 19-NE