

# WADA Technical Document – ISL TD2027NA

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

### 1.0 Introduction

This *Technical Document (TD)*, which constitutes an integral part of the *International Standard for Laboratories (ISL* <sup>[1]</sup>), 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 <sup>[2-4]</sup>, and ii) in situ microbial degradation of androsterone (A) to 19-NA <sup>[5]</sup>. 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 <sup>[4, 6-12]</sup>.]*

### 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<sup>n</sup>) 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<sup>n</sup> identification (as per ISL TD IDCR <sup>[13]</sup>) 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<sup>n</sup>, 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  $\beta$ -D-glucuronide linkages (e.g., purified  $\beta$ -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<sup>n</sup> 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 <sup>[14]</sup>.
  - 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 ( $\leq$ ) 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  $\beta$ -D-glucuronide linkages (e.g., purified  $\beta$ -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 ( $\leq$ ) 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., <sup>2</sup>H- or <sup>13</sup>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,

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## 3.2 Confirmation Procedure

### 3.2.1 “A” Sample Confirmation Procedure

The “A” CP of 19-norsteroids includes the GC-MS<sup>n</sup> estimation of the concentration and identification (in compliance with the ISL *TD* IDCR <sup>[13]</sup>), 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<sup>n</sup> Confirmation Procedure

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

- a) The Limit of Identification (LOI) of the GC-MS<sup>n</sup> 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<sup>n</sup> 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<sup>n</sup> 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 <sup>[15]</sup>, including the use of:
  - i. A PQC urine sample at the *MRL* of 15 ng/mL.
  - ii. A Single Point Calibrator (SPC) <sup>1</sup> 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 ( $\leq$ ) 1.25 ng/mL.
  - iv. An appropriate labeled ISTD (e.g., <sup>2</sup>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<sup>n</sup> 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.]*

<sup>1</sup> 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.

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## 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 5 $\alpha$ -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	<ul style="list-style-type: none"> <li>• &gt; 15 ng/mL<sup>2,3</sup> (AAF)</li> </ul>	<ul style="list-style-type: none"> <li>• Males</li> <li>• Females               <ul style="list-style-type: none"> <li>– Non-pregnant, and</li> <li>– Absence of 3,5-tetrahydronorethisterone (THNE)</li> </ul> </li> </ul>
	<ul style="list-style-type: none"> <li>• ≤ 15 ng/mL<sup>2,3</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Females               <ul style="list-style-type: none"> <li>– Presence of THNE</li> </ul> </li> </ul>
Required	<ul style="list-style-type: none"> <li>• 2.5 - 15 ng/mL<sup>2,4</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Males</li> <li>• Females               <ul style="list-style-type: none"> <li>– Non-pregnant, and</li> <li>– Absence of THNE</li> </ul> </li> </ul>
	<ul style="list-style-type: none"> <li>• &gt; 15 ng/mL<sup>2,3</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Females               <ul style="list-style-type: none"> <li>– Pregnant females</li> </ul> </li> </ul>
Optional	<ul style="list-style-type: none"> <li>• 2.5 - 15 ng/mL<sup>4</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Females               <ul style="list-style-type: none"> <li>– Pregnant females</li> </ul> </li> </ul>
	<ul style="list-style-type: none"> <li>• &lt; 2.5 ng/mL</li> </ul>	<ul style="list-style-type: none"> <li>• Males</li> <li>• Females               <ul style="list-style-type: none"> <li>– Non-pregnant</li> <li>– Absence of THNE</li> </ul> </li> </ul>

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<sup>2</sup> According to the ISL *TD MRL* <sup>[15]</sup>, 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.

<sup>3</sup> 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<sup>n</sup> 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*) <sup>[15]</sup>, the estimated 19-NA concentration shall be adjusted for the SG of 1.020 <sup>[15]</sup>. 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.

<sup>4</sup> 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* <sup>[16]</sup> 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.*,  $|\Delta\delta^{13}C| = |\delta^{13}C_{ERC} - \delta^{13}C_{19-NA (or 19-NE)}|$ , shall be greater than (>) 3.0 ‰ (refer also to the ISL *TD IRMS* <sup>[16]</sup> 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 <sup>[16]</sup> 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.*,  $\delta^{13}C_{19-NA}$  between -16.000 ‰ and -24.000 ‰) <sup>[11, 17]</sup>, 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

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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 ( $\geq$ ) 2.5 ng/mL in the urine *Sample* of a female *Athlete*, the Laboratory shall:

- Test for pregnancy based on the measurement of urinary human Chorionic Gonadotrophin (hCG) in the urine *Sample*.
- 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  $\leq$  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 (Eti), 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

- 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.
- 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].

# WADA Technical Document – ISL TD2027NA

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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 <sup>[16]</sup> for GC/C/IRMS results reporting requirements.

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

<b>Sample Origin</b>	<b>Adverse Analytical Finding</b>	<b>Atypical Finding</b>	<b><u>Negative Finding</u></b>
<b>4.1 Pregnant Female Athletes</b>	<b>Results</b>		
	Results of the GC/C/IRMS analysis are consistent with the exogenous origin of 19-NA.	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA &gt; 15 ng/mL. <sup>2,3</sup></li> <li>AND</li> <li>- Results of GC/C/IRMS analysis are inconclusive or do not meet the criteria supporting an exogenous origin of 19-NA.</li> </ul>	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA ≤ 15 ng/mL. <sup>2,3</sup></li> <li>AND</li> <li>- GC/C/IRMS analysis is either not performed, or the results are consistent with an endogenous origin of 19-NA.</li> </ul>
	<b>Test Report (No reference to the pregnancy status of the Athlete shall be reported)</b>		
	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA, expressed as “≤15 ng/mL” or “&gt;15 ng/mL” <sup>2,3</sup>.</li> <li>- 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 δ<sup>13</sup>C values for 19-NA and the ERCs, as well as the respective <i>u<sub>c</sub></i> (‰).</li> </ul>	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA &gt;15 ng/mL <sup>2,3</sup>.</li> <li>- 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 δ<sup>13</sup>C values for 19-NA and the ERCs as well as the respective <i>u<sub>c</sub></i> (‰).</li> </ul>	<ul style="list-style-type: none"> <li>- If GC/C/IRMS analysis is performed:</li> <li>- Estimated concentration of 19-NA ≤ 15 ng/mL <sup>2,3</sup>.</li> <li>- Include a comment indicating that the GC/C/IRMS finding is consistent with an endogenous origin of 19-NA, the δ<sup>13</sup>C values for 19-NA and the ERC, as well as the respective <i>u<sub>c</sub></i> (‰).</li> </ul>

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Sample Origin	Adverse Analytical Finding	Atypical Finding	Negative Finding
<b>4.2 Female Athletes using Norethisterone</b>	<b>Results</b>		
	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA &gt; 15 ng/mL <sup>2,3</sup>.</li> <li>- The estimated concentration ratio of 19-NA/THNE is &gt; 2.</li> </ul>	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA &gt; 15 ng/mL <sup>2,3</sup>.</li> <li>- The estimated concentration ratio of 19-NA/THNE is ≤ 2.</li> </ul>	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA ≤ 15 ng/mL <sup>2,3</sup></li> </ul>
	<b>Test Report</b>		
	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA &gt;15 ng/mL <sup>2,3</sup>.</li> <li>- A comment describing the presence of THNE in the <i>Sample</i>.</li> </ul> <p><i>Example:</i></p> <p><i>“19-NA was found in the Sample at an estimated concentration &gt; 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”.</i></p>	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA &gt;15 ng/mL <sup>2,3</sup>.</li> <li>- A comment describing the presence of THNE in the <i>Sample</i>.</li> </ul> <p><i>Example:</i></p> <p><i>“19-NA was found in the Sample at an estimated concentration &gt; 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”</i></p>	<p><b>No reference to the use of norethisterone shall be reported.</b></p>
<b>4.3 Male or Female Athletes (neither pregnant nor using norethisterone)</b>	<b>Results</b>		
	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA &gt; 15 ng/mL. <sup>2,3</sup></li> <li>OR</li> <li>- Estimated concentration of 19-NA ≤ 15 ng/mL <sup>2,3</sup> and GC/C/IRMS results are consistent with an exogenous origin of 19-NA.</li> </ul>	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA ≤ 15 ng/mL <sup>2,3</sup> and GC/C/IRMS results are inconclusive or do not meet the criteria supporting an exogenous origin of 19-NA.</li> <li>OR</li> </ul>	<ul style="list-style-type: none"> <li>- Estimated concentration of 19-NA &lt; 2.5 ng/mL (and too low to perform GC/C/IRMS analysis).</li> <li>OR</li> <li>- Estimated concentration of 19-NA between 2.5 - 15 ng/mL <sup>2,3</sup> and GC/C/IRMS</li> </ul>

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<b>4.3 Male or Female Athletes (neither pregnant nor using norethisterone) (cont.)</b>	<p>OR</p> <ul style="list-style-type: none"> <li>- 19-NA cannot be reliably analyzed in the <i>Sample</i> (e.g., due to the presence of 5<math>\alpha</math>-reductase inhibitor activity) and the GC/C/IRMS results for 19-NE are consistent with an exogenous origin of 19-NE.</li> </ul>	<ul style="list-style-type: none"> <li>- 19-NA cannot be reliably analyzed in the <i>Sample</i> (e.g., due to the presence of 5<math>\alpha</math>-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.</li> </ul>	<p>results are consistent with an endogenous origin of 19-NA.</p> <p>OR</p> <ul style="list-style-type: none"> <li>- 19-NA cannot be reliably analyzed in the <i>Sample</i> (e.g., due to the presence of 5<math>\alpha</math>-reductase inhibitor activity) and the GC/C/IRMS results for 19-NE are consistent with an endogenous origin of 19-NE.</li> </ul>	
	<b>Interpretation of Results</b>			
	<ul style="list-style-type: none"> <li>- If 19-NA &gt; 15 ng/mL with no GC/C/IRMS analysis: <ul style="list-style-type: none"> <li>• Estimated concentration of 19-NA &gt;15 ng/mL <sup>2,3</sup>.</li> <li>• For <i>Samples</i> from female <i>Athletes</i>, include a comment explaining that pregnancy and the use of norethisterone were excluded as the source of 19-NA.</li> </ul> <p><i>Example: “The 19-NA finding is not consistent with pregnancy or the use of norethisterone”.</i></p> </li> <li>- If 19-NA ≤ 15 ng/mL with positive GC/C/IRMS results <ul style="list-style-type: none"> <li>• Estimated concentration of 19-NA ≤ 15 ng/mL.</li> <li>• 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 <math>\delta^{13}\text{C}</math> values for 19-NA</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- If 19-NA ≤ 15 ng/mL with inconclusive GC/C/IRMS results: <ul style="list-style-type: none"> <li>• Estimated concentration of 19-NA ≤ 15 ng/mL <sup>2,3</sup>.</li> <li>• 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 <math>\delta^{13}\text{C}</math> values for 19-NA and the ERCs as well as the respective <math>u_c</math> (‰).</li> <li>• For <i>Samples</i> from female <i>Athletes</i>, clarify that pregnancy was excluded as the source of 19-NA</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- If GC/C/IRMS analysis is performed: <ul style="list-style-type: none"> <li>• Estimated concentration of 19-NA (either &lt; 2.5 ng/mL or between 2.5-15 ng/mL).</li> <li>• 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 <math>\delta^{13}\text{C}</math> values for 19-NA and ERC as well as the respective <math>u_c</math> (‰).</li> </ul> </li> <li>- If the finding is based on negative GC/C/IRMS results for 19-NE: <ul style="list-style-type: none"> <li>• 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 <math>\delta^{13}\text{C}</math> values for 19-NE and the ERC as</li> </ul> </li> </ul>	

# WADA Technical Document – ISL TD2027NA

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<p><b>4.3 Male or Female Athletes (neither pregnant nor using norethisterone) (cont.)</b></p>	<p>and the ERCs as well as the respective <math>u_c</math> (‰).</p> <ul style="list-style-type: none"> <li>• For <i>Samples</i> from female <i>Athletes</i>, 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”).</li> </ul> <p>– If the finding is based on positive GC/C/IRMS results for 19-NE:</p> <p>– 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 <math>\delta^{13}\text{C}</math> values for 19-NE and the ERCs as well as the respective <math>u_c</math> (‰).</p>	<p><i>Example: “The 19-NA finding is not consistent with pregnancy”.</i></p> <ul style="list-style-type: none"> <li>• Recommend the <u>TA</u> (or <u>RMA</u>, if different) to conduct follow-up no-notice tests on the <i>Athlete</i> as soon as possible and evaluate the kinetics of 19-NA excretion in urine.</li> </ul> <p>– If the finding is based on inconclusive GC/C/IRMS results for 19-NE:</p> <p>– 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 <math>\delta^{13}\text{C}</math> values for 19-NE and the ERCs as well as the respective <math>u_c</math> (‰).</p>	<p>well as the respective <math>u_c</math> (‰).</p>
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# WADA Technical Document – ISL TD2027NA

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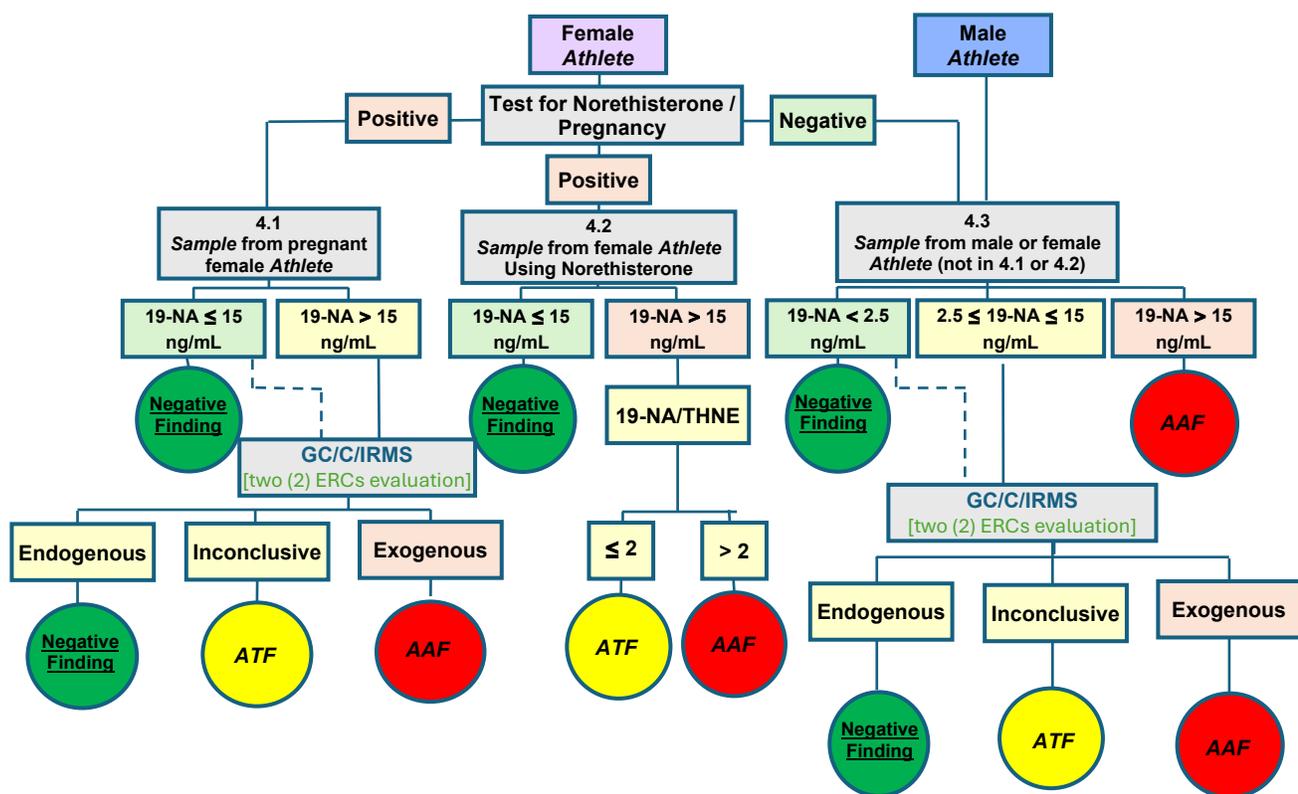
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## ANNEX A. Confirmation Procedure for 19-Norsteroids

### A1. Confirmation Procedure for 19-NA



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

