

WADA Technical Document – TD2009NA

| | | | |
|------------------|---------------------------|-----------------|--------------------------|
| Document Number: | TD2009NA | Version Number: | 1.0 |
| Written by: | WADA Laboratory Committee | Approved by: | WADA Executive Committee |
| Date: | September 15, 2009 | Effective Date: | Jan 01, 2010 |

HARMONIZATION OF ANALYSIS AND REPORTING OF 19-NORSTEROIDS RELATED TO NANDROLONE

1.0 Introduction

This document has been established to harmonize the analysis and reporting of 19-norsteroids related to nandrolone as *Adverse Analytical Findings* by Laboratories.

The detection of the use of nandrolone and other 19-norsteroids is based primarily upon the identification of the main urinary metabolite, 19-norandrosterone (19-NA) in an amount greater than 2 ng/mL. More than one metabolite (e.g., 19-noretiocholanolone (19-NE)) of administered norsteroids may be detected and reported but the identification and quantification of the 19-NA metabolite only (derived from hydrolysis with β -glucuronidase) is sufficient to report an *Adverse Analytical Finding*.

2.0 Analysis

2.1 Identification and Quantification

In addition to meeting the identification criteria described in the IDCR Technical Document, the Laboratory shall demonstrate that the concentration of 19-NA is above the threshold as set out in the MRPL Technical Document.

The quantification method used to calculate the concentration of 19-NA shall include or have the following characteristics:

- a deuterated internal standard (d_4 -19-NA-glucuronide is the preferred internal standard since it corrects for both the hydrolysis and other analytical steps);
- a calibration curve at an appropriate range bracketing the estimated concentration of the analyte;
- the use of appropriate quality control samples. For example, a negative control (without the presence of 19-NA or at a concentration \leq 1ng/mL of 19-NA) and a positive control in the range of 3 to 5 ng/mL of 19-NA may be used. Alternatively, a freeze-dried urine reference material with approximately 2 ng/mL of 19-NA may be used (e.g., NMI Reference number MX002).
- the expanded measurement uncertainty established by the Laboratory shall be less than ± 0.4 ng/mL at the threshold.

2.2 Additional mandatory tests

The Laboratory shall also perform methods to test for pregnancy (e.g. hCG) and detection of tetrahydronorethisterone in urine *Samples* from female *Athletes* that have 19-NA concentrations greater than the threshold.

WADA Technical Document – TD2009NA

| | | | |
|------------------|---------------------------|-----------------|--------------------------|
| Document Number: | TD2009NA | Version Number: | 1.0 |
| Written by: | WADA Laboratory Committee | Approved by: | WADA Executive Committee |
| Date: | September 15, 2009 | Effective Date: | Jan 01, 2010 |

In extremely rare circumstances, 19-NE, and to a lesser extent 19-NA, can be formed by 19-demethylation of abundant endogenous steroids. These “unstable” *Samples* show the presence of low and comparable levels of 19-NA and 19-NE where the ratio of 19-NA/19-NE is less than the ratio of A/E (androsterone /etiocholanolone).

When concentrations less than 10 ng/mL of 19-NA are measured and the *Sample* shows the above feature of instability, the Laboratory shall perform a stability test. The stability test, which incorporates deuterated androsterone and etiocholanolone, has been described by Grosse et al [1].

- If the stability test is positive (ie, *Sample* is “unstable”), the 19-NA result shall not be reported.
- If the stability test is negative, the *Sample* shall have a GC/C/IRMS analysis performed on the 19-NA [2]. The criterion for an unstable urine by GC/C/IRMS shall be a difference of less than 3.0‰¹ between the measured δ values ($\Delta\delta = \delta_{\text{Androsterone}} - \delta_{19\text{-NA}}$) of 19-NA and androsterone. The GC/C/IRMS analysis should include the confirmation of the 19-NA peak identity (for example by GC/MS analysis).
- If the stability test is negative and the GC/C/IRMS test shows that the 19-NA is endogenous, the 19-NA result shall not be reported.
- If the stability test is negative and GC/C/IRMS test shows that the 19-NA is exogenous, the result shall be reported as an *Adverse Analytical Finding*.

3.0 Reporting

The Laboratory shall report the finding of 19-NA in a urine *Sample* from a male or a female *Athlete* at a concentration above the decision limit (DL) after adjustment of the threshold for specific gravity (see section 3.1 and 3.2 below) and meeting the requirements of IDCR TD as defined below:

- A. *Samples* from male or non-pregnant female *Athletes* for which 19-NA concentrations are determined to be greater than 25 ng/mL shall be reported as an *Adverse Analytical Finding* simply as “>25 ng/mL” without the need for reporting concentration.
- B. *Samples* from male or non-pregnant female *Athletes* for which 19-NA concentrations are determined to be between 10 ng/mL and 25 ng/mL shall be reported as an *Adverse Analytical Finding* as the mean concentration from triplicate determinations to not more than two significant figures² as well as the expanded measurement uncertainty at

¹ The difference between δ values need not be corrected for any derivatizing agent since both androsterone and norandrosterone would have the same contribution from added carbon atoms.

² A result between 2 and 10 would be reported, for example, as “5.1 ng/mL”. A result between 10 and 25 would be reported as “17 ng/mL”.

WADA Technical Document – TD2009NA

| | | | |
|------------------|---------------------------|-----------------|--------------------------|
| Document Number: | TD2009NA | Version Number: | 1.0 |
| Written by: | WADA Laboratory Committee | Approved by: | WADA Executive Committee |
| Date: | September 15, 2009 | Effective Date: | Jan 01, 2010 |

the 95% confidence level ($k=2$; two-sided). A comment or opinion shall be added according to 3.2 below.

- C. *Samples* from male or non-pregnant female *Athletes* not using norethisterone for which 19-NA concentrations are determined to be between the DL and 10 ng/mL and for which the features of an “unstable” urine are NOT met shall be reported as an *Adverse Analytical Finding* as the mean concentration from triplicate determinations to not more than two significant figures as well as the expanded measurement uncertainty at the 95% confidence level ($k=2$; two-sided). A comment or opinion shall be added according to 3.2 below.
- D. *Samples* from male or non-pregnant female *Athletes* not using norethisterone for which 19-NA concentrations are determined to be between the DL and 10 ng/mL and for which the features of an “unstable” urine are met shall be reported as Negative.
- E. *Samples* from non-pregnant female *Athletes* using norethisterone shall be reported as *Adverse Analytical Finding* but adding a comment or opinion about the use of norethisterone as described below under section 3.3.3. The presence of tetrahydronorethisterone should be confirmed.
- F. *Samples* from pregnant female *Athletes* for which 19-NA concentrations are determined to be greater than the DL shall be reported as Negative unless IRMS testing is consistent with exogenous administration. The Laboratory shall include in the report a comment indicating the test results that support the opinion of the pregnancy as described below under section 3.3.

3.1 Adjusted Thresholds

Only in the case of urine *Samples* measured with a specific gravity above 1.020 (in the Laboratory), an adjustment to the threshold shall be made to take into account the specific gravity of the *Sample* (since higher 19-NA concentrations have been associated with higher specific gravities (e.g. [3])), using the following formula:

$$\text{Threshold}_{\text{adjusted}} = [(\text{Specific gravity of the Sample} - 1) / (1.020 - 1)] \times 2\text{ng/mL}$$

3.2 Decision limit for 19-NA

A decision limit (DL)³ shall be established by adding the expanded measurement uncertainty determined from inter-laboratory WADA External Quality Assessment Scheme (EQAS) test results to the adjusted threshold. For the DL, $k_{0.95} = 1.645$ (one sided) shall be used. Based on this determination, a guard band of 0.4 ng/mL⁴

³ Eurachem/CITAC (2007) Use of uncertainty information in compliance assessment.

⁴ The combined standard measurement uncertainty based on the WADA EQAS testing results at the 2 ng/mL threshold has been estimated as 0.2 ng/mL. The expanded uncertainty based on the one-sided $k_{0.95}$ would be 0.33 which has been rounded up to 0.4 ng/mL.

WADA Technical Document – TD2009NA

| | | | |
|------------------|---------------------------|-----------------|--------------------------|
| Document Number: | TD2009NA | Version Number: | 1.0 |
| Written by: | WADA Laboratory Committee | Approved by: | WADA Executive Committee |
| Date: | September 15, 2009 | Effective Date: | Jan 01, 2010 |

shall be added to the adjusted threshold to determine the DL for an individual 19-NA test result⁵. The DL (after adjustment of the threshold for specific gravity) shall be included on the Laboratory test report. The DL shall be used to determine whether an *Adverse Analytical Finding* is reported for the *Sample*.

The result from a *Sample* shall be reported as an *Adverse Analytical Finding* if the measured mean concentration is greater than the DL unless a *Sample* meets one of the conditions discussed in sections 3.3 and 3.4 below. If the *Sample* does meet one of the conditions discussed in 3.3 and 3.4 below, then it shall be reported as an *Atypical Finding* and both the Testing Authority and WADA shall be notified of the results as a comment in the test report.

3.3 Female Athlete's Samples

When 19-NA exceeds the DL in the urine *Sample* of a female *Athlete*, the Laboratory shall include in the test report the results of tests to determine whether the 19-NA is due to pregnancy or to the intake of a medication containing norethisterone.

3.3.1 If hCG is present at a concentration less than 1000 IU/L, a comment or opinion shall be added to the test report indicating that the test was performed and that the 19-NA result was not consistent with pregnancy. An example would be:

"The Sample was analyzed for hCG and the concentration was less than 1000 mIU/mL; indicating that the 19-NA finding is not the result of pregnancy."

3.3.2 If another compound, such as pregnanediol, is used to detect pregnancy, a comment or opinion shall be added to the test report indicating that the test was performed and that the result was not consistent with pregnancy.

3.3.3 If tetrahydronorethisterone (metabolite of norethisterone contraceptive) is not detected in the urine *Sample*, a comment or opinion shall be added to the test report indicating that the test was performed and that norethisterone was not present. An example would be:

"The Sample was found not to contain tetrahydronorethisterone by GC/MS analysis; indicating that the finding is not the result of an administration of a norethisterone contraceptive."

3.4 19-demethylation

In the rare event that a *Sample* is found to meet the common features of an "unstable" urine and therefore tested as in Section 2.2 above, the Laboratory shall include the results of the stability test(s) in the test report for the 19-NA *Adverse Analytical Finding*. Therefore, a comment shall be on the test report indicating that the stability test was performed and that the *Sample* is stable. In addition, the results of the GC/C/IRMS analysis, including the δ values for 19-NA and androsterone and the $\Delta\delta$ value shall be included in the test report.

⁵ Thus the Decision Limit for the 19-NA test result for a *Sample* with a specific gravity of 1.020 or less is 2.4 ng/mL. When the specific gravity of a *Sample* is 1.030, for example, the adjusted threshold is 3.0 ng/mL and DL shall be 3.4 ng/mL.

WADA Technical Document – TD2009NA

| | | | |
|------------------|---------------------------|-----------------|--------------------------|
| Document Number: | TD2009NA | Version Number: | 1.0 |
| Written by: | WADA Laboratory Committee | Approved by: | WADA Executive Committee |
| Date: | September 15, 2009 | Effective Date: | Jan 01, 2010 |

4.0 References

1. Grosse J, Anielski P, Hemmersbach P, Lund H, Mueller RK, Rautenberg C, Thieme D. Formation of 19-norsteroids by in situ demethylations of endogenous steroids in stored urine samples. *Steroids* 2005; 70: 499-506.
2. Hebestreit M, Flenker U, Fusshöller G, Geyer H, Güntner U, Mareck U, Piper T, Thevis M, Ayotte C, Schänzer W Determination of the origin of urinary norandrosterone traces by gas chromatography combustion isotope ratio mass spectrometry. *Analyst* 2006; 131:1021-6.
3. Walker CJ, Cowan DA, James VHT, Lau JCY, Kicman AT. Doping in sport—1. Excretion of 19-norandrosterone by healthy women, including those using contraceptives containing norethisterone. *Steroids* 2009;74:329-35.