Document Number:	TD2004NA	Version Number:	1.0
Written by:	WADA Laboratory Committee	Approved by:	WADA Executive Committee
Date:	28 May, 2004	Effective Date:	13 August, 2004

REPORTING NORANDROSTERONE FINDINGS

1. Introduction:

This document has been established to harmonize analysis and reporting of norandrosterone *Adverse Analytical Findings* by Laboratories.

The administration of 19-norsteroids such as 19-nortestosterone (nandrolone), 19-norandrostene-3,17-dione and 19-norandrostene-3,17-diol (delta-4 and -5 isomers) has been shown to lead mainly to the excretion of 19-norandrosterone (NA), 19-noretiocholanolone (NE) and 19-norepiandrosterone (NEA). The latter is found exclusively as its sulfoconjugate while the others are usually excreted as their glucuronide derivative. The sulfate derivatives, generally persistent, may be prevalent at the end of the excretion period.

After the i.m. administration of the long-lasting preparations of nandrolone, the metabolites may be detected for months, but metabolites formed after the oral ingestion are excreted massively in the first hours and remain detectable for only a few days. The excretion of 19-norandrosterone generally predominates that of the 5β-isomer but inversed proportions have been reported in some individuals after oral administration either at the end of the excretion period or when ?^{5}-isomers of related norsteroids were taken (1). Norandrosterone is excreted during pregnancy and as a minor metabolite of norethisterone (2).

Special procedures such as more sensitive instrumentation, larger volumes of urine and more extensive sample clean-up were needed to detect, identify and quantify endogenous 19-norandrosterone (with limits of detection needing to be ten times lower than routine testing i.e. around 0.01 ng/mL). Under tightly controlled conditions, when 19-norandrosterone was detected in male specimens, it was found at mean values of less than 0,1 ng/mL which is well below the limit for reporting *Adverse Analytical Findings* (3). The physiological levels of 19-norandrosterone measured in samples collected from females are lower than 1 ng/mL, a maximum value of 0.8 ng/mL having been recorded during ovulation and correlates apparently with high levels of estrogens (4).

It appears that exercise does not increase physiological levels of 19-norandrosterone significantly and certainly not sufficiently to approach the threshold (5). A few urine specimens collected from sportsmen after the competition were reported to contain 19-norandrosterone in an amount approaching 1 to 2 ng/mL. However, these observations were made without adequate controls to exclude possible administration of norsteroids (6).

Document Number:	TD2004NA	Version Number:	1.0
Written by:	WADA Laboratory Committee	Approved by:	WADA Executive Committee
Date:	28 May, 2004	Effective Date:	13 August, 2004

Although highly improbable, the intake of a substantial amount of non-castrated pig offal, in which the presence of norsteroids such as 19-nortestosterone has been demonstrated, could result in the excretion of 19-norandrosterone in an amount above the threshold during a few hours after ingestion (7).

Finally, the administration of some nutritional "supplements" can be the source of the presence of 19-norandrosterone in human urine samples (8).

2. Reporting requirements

The following requirements shall be applied by all Laboratories in their routine practice.

The Laboratory is to report as an *Adverse Analytical Finding*, any urine *Sample* from either a male or a female containing 19-norandrosterone (19-NA) at a concentration greater than 2 ng/mL. The specific gravity of the *Sample* is to be equal to or lower than 1.020 (measured in the Laboratory using an appropriate instrument). For urine *Samples* with a specific gravity above 1.020 a correction to the threshold is to be made.

The correction of the threshold to take into account the specific gravity of the *Sample* will be calculated using the following formula:

$$\text{Threshold}_{1.020}\ \text{ng/mL} = (\text{Specific gravity}_{\text{ of the } \textit{Sample}} - 1) / (1.020 - 1) \bullet 2\ \text{ng/mL}$$

In addition to meeting the identification criteria (TD2003IDCR) the Laboratory must demonstrate that the concentration of 19-NA is above the threshold. The concentration of 19-norandrosterone must also be determined when it is lower than 10 ng/mL. The estimated expanded uncertainty must be considered for reporting.

More than one metabolite of administered norsteroids may be detected, but only the identification and quantification of 19-NA and its glucuronide (calculated as the total following hydrolysis of the glucuronide) is sufficient to report an *Adverse Analytical Finding*.

Before reporting an *Adverse Analytical Finding* in the urine *Sample* of a female, the Laboratory must take steps to ascertain that the presence of low levels of 19-norandrosterone is not due to pregnancy or to the intake of a birth control preparation or progestogen medication containing norethisterone. The Laboratory must document the absence of hCG i.e. less than 5 mIU/mL of immunoreactive hCG to exclude the possibility that an *Adverse Analytical Finding* had arisen because of pregnancy. The Laboratory will determine whether it is reasonable that the 19-norandrosterone was excreted in the amount measured consequent to the intake of norethisterone, by verifying that the major isomer of

glucuroconjugated tetrahydronorethisterone is present. The Laboratory will in such a case add the following phrase to the report "could be compatible with a norethisterone treatment".

The official text of the technical document on the Reporting Norandrosterone Findings shall be maintained by *WADA* and shall be published in English and French. In the event of any conflict between the English and French versions, the English version shall prevail.

3. References:

1. Engel LL, Alexander J, Wheeler M. *Urinary metabolites of administered 19-nortestosterone*. J Biol Chem 1958;231:159-64;

Massé R, Laliberté C, Tremblay L, Dugal R. *Gas chromatographic/mass spectrometric analysis of 19-nortestosterone urinary metabolites in man*. Biomed Mass Spectrom 1985; 12(3):115-21;

Schänzer W. *Metabolism of anabolic androgenic steroids*. Clin Chem 1996; 42(7): 1001-20;

Kintz P, Cirimele V, Ludes B. *Norandrostérone et norétiocholanolone: les métabolites révélateurs*. Acta Clin Belg Suppl 1999;Suppl 1:68-73

Schänzer W, Breidbach A, Geyer H, van Kuk C, Nolteernsting E, Thevis M. *Metabolism of nortestosterone, norandrostenedione and norandrostenediol. Identification of 3a-hydroxyestr-4-en-17-one glucuronide and 3a,16a-dihydroxy-5a-estran-17-one glucuronide and sulphate*. In: Schänzer W, Geyer H, Gotzmann A and Mareck-Engelke U, editors. Recent advances in doping analysis (7). 17th Cologne Workshop on Dope Analysis 14th to 19th March 1999. Köln: Sport and Buch Strauss, 2000. pp. 155-74;

Uralets VP, Gillette PA. *Over-the-Counter anabolic steroids 4-androsten-3,17-dione; 4-androsten-3a,17a-diol, and 19-nor-4-androstene-3,17-dione: Excretion studies in men.* J Anal Toxicol 1999;23(5):357-66;

Uralets VP, Gillette PA. *Over-the-Counter delta-5 anabolic steroids 5-androsten-3,17-dione; 5-androsten-3a,17b-diol; Dehydroepiandrosterone and 19-Nor-5-androstene-3,17-dione: Excretion studies in men*. J Anal Toxicol 2000;24 (3):188-93.

2. Dehennin L, Jondet M, Scholler R. *Androgen and 19-norsteroid profiles in human preovulatory follicles from stimulated cycles: an isotope dilution-mass spectrometric study*. J Steroid Biochem 1987; 26(3):399-405.;

Reznik Y, Herrou M, Dehennin L, Lemaire M, Leymarie P. *Rising plasma levels of 19-nortestosterone throughout pregnancy: determination by radioimmunoassay and validation by gas chromatography-mass spectrometry*. J Clin Endocr Metabol 1987; 64(5):1086-8;

Van Eenoo P, Delbeke FT, de Jong FH, De Backer P., *Endogenous origin of norandrosterone in female urine: indirect evidence for the production of 19- norsteroids as by-products in the conversion from androgen to estrogen*, J. Steroid Biochem. Mol. Biol., 78 (4) (2001) 351-7.

3. Ciardi M, Ciccoli R, Barbarulo MV, Nicoletti R. *Presence of norandrosterone in "normal" urine samples*. In: Schänzer W, Geyer H, Gotzmann A and Mareck-Engelke U, editors. Recent advances in doping analysis (6). 16th Cologne Workshop on Dope Analysis 15th to 20th March 1998. Köln: Sport and Buch Strauss, 1999. pp. 97-104.

Jeanneau T, Kintz P, Cirimele V, Ludes B. *Détermination des concentrations physiologiques de la norandrostérone et de la norétiocholanolone, métabolites urinaires de la nandrolone par CPG/SM*. Toxicorama 1999;XI:25-9;

Dehennin, L. et al., *Urinary excretion of 19-norandrosterone of endogenous origin in man: quantitative analysis by gc/ms*, J. Chromatogr. B 721 (1999) 301;

Le Bizec, B., Monteau, F., Gaudin, I., and André, F. (1999). *Evidence for the presence of endogenous 19-norandrosterone in human urine*. J. Chromatogr. B. 723: 157 – 172

4. Van Eenoo P, Delbeke FT, De Jong FH, De Backer P. *Endogenous origin of norandrosterone in female urine: indirect evidence for the production of 19-norsteroids as by-products in the conversion from androgen to estrogen*. J Steroids Biochem Mol Biol 2001;78(4):351-7;

Hemmersbach P, Hågensen AH, Misund J. *Determination of urinary norandrosterone excretion in females during one menstrual cycle by gas chromatography/mass spectrometry*. In: Schänzer W, Geyer H, Gotzmann A and Mareck-Engelke U, editors. Recent

advances in doping analysis (7). 17th Cologne Workshop on Dope Analysis 14th to 19th March 1999. Köln: Sport and Buch Strauss, 2000. pp. 141-4.

5. Schmitt, N., et al., *Nandrolone excretion is not increased by exhaustive exercise in trained athletes*, Med. Sci. Sports & Exerc., 34, 1436 (2002)

6. Robinson N., Taroni F., Saugy M., Ayotte C., Mangin P. and Dvorak J., *Detection of nandrolone metabolites in urine after a football game in professional and amateur players: a Bayesian comparison*, Forensic Science International, 122 (2001) 130;

Le Bizec B. et al., *Endogenous Nandrolone metabolites in human urine. Two-year monitoring of male professional soccer players*, J. Anal. Toxicol., 26 (2002) 43;
Gambelunghe C, Sommavilla M, Rossi R., *Testing for nandrolone metabolites in urine samples of professional athletes and sedentary subjects by GC/MS/MS analysis*, Biomed. Chromatogr. 16(8) (2002) 508-12

7. Le Bizec, B., I. Gaudin, F. Monteau, F. André, S. Impens, K. De Wasch and H. De Brabander, *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 (2000);

C. Ayotte, C. Guay, M. Cléroux, D. Goudreault and A. Fakirian, *Origin of elevated levels of norandrosterone in human urine: half-truths vs facts*, Lecture presented during the 2002 Workshop for Doping analysis, in Recent Advances of Doping Analysis, (2002), p. 13

8. Ayotte, C., *Nutritional supplements and doping controls*, IAF New Studies in Athletics, 14 (1999) 37;

Ayotte C. et al., Sport *Nutritional Supplements: Quality and Doping Controls*, Canadian J. Appl. Physiology (2001), 2b, Supplement, 120-129;

Kamber, M., Baume, N., Saugy, M., and Rivier, L. (2000). *Nutritional supplements as a source for positive doping cases?*. J. Int. Sport Nutr. Exerc. Metab. 11: 258;

de Cock, K.J. et al., *Detection and determination of anabolic steroids in nutritional supplements*, J. Pharm. Biomed. Anal., 25: 843 (2001).

Geyer H. et al., *The analysis of « non-hormonal » nutritional supplements for prohormones*, in Recent advances in Doping analysis, Proceedings of the 19th Cologne

Workshop on Dope Analysis, March 2001, p. 63; and Report from IOC funded study available at http://multimedia.olympic.org/pdf/en_report_324.pdf

Reti S., *Steroids masquerading as natural herbs: a case for regulatory control*. N Z Med J. 2002;115(1159):U125.

Catlin DH, Leder BZ, Ahrens B, Starcevic B, Hatton CK, Green GA, Finkelstein JS., *Trace contamination of over-the-counter androstenedione and positive urine test results for a nandrolone metabolite*, JAMA. 2000; 284(20):2618-21.

UK Sports, *Nandrolone report* and *Nandrolone progress report to the UK Sports Council from the expert committee on nandrolone*, January 2001 and February 2003.