

EXPLANATORY TECHNICAL NOTE**Stability of 19-norandrosterone findings in urine**

In 2004, D. Thieme et al.¹ reported the formation of 19-noretiocholanolone (19-NE) and 19-norandrosterone (19-NA) in some athletes' urine samples following incubation. Other groups recently confirmed this finding. This new phenomenon is extremely rare and appears to occur in particular conditions. At present, ongoing scientific investigations have not determined the origin of this phenomenon and therefore extra precautions have been taken to ensure that all evaluation criteria mentioned hereunder encompass a sufficient safety margin. Such level may be reduced in the coming months with the knowledge gathered from research which is presently being conducted.

The formation of 19-NE and 19-NA is observed under particular conditions and in extremely rare urine samples exhibiting as common features, the presence of low and comparable levels of 19-NA and 19-NE where $19\text{-NA}/19\text{-NE} < A/E$, and turbidity. Also, as an additional indication, in most cases, the specific gravity is high ($> 1,020$ ²). To be extremely cautious we consider that it is possible that this phenomenon may be observed for sample where the 19-NA concentration is lower than 10 ng/mL. However, the low levels of 19-NA observed in such unstable urines are ranging from 0.1 to only a maximum of 5.4 ng/mL³ (corrected for a specific gravity of 1.020). The 19-demethylation of etiocholanolone (E) and androsterone (A) is observed after incubation of those specimens. The reaction, favouring the 5 β - over the 5 α -isomer, has been shown to depend on the temperature e.g., incubations at 37°C producing higher yields, and on the concentration of substrate.

When all the common features of "unstable" urine described are not observed and in any case when the level of 19-NA is above 10 ng/mL, adverse analytical findings shall be reported according to TD2004NA.

Urine samples below 10 ng/mL of 19-NA and exhibiting all of the common features described above shall be submitted to a stability test before reporting an adverse analytical finding.

¹ D. Thieme, P. Anielski, J. Grosse, P. Hemmersbach, H. Lund and C. Rautenberg, *Kinetic of in-situ demethylation of endogenous steroids in urine samples*, in: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (12). Sport und Buch Strauß, Köln (2004) 177-188; J. Grosse, P. Anielski, P. Hemmersbach, H. Lund, R.K. Mueller, C. Rautenberg and D. Thieme, *Formation of 19-norsteroids by in-situ demethylation of endogenous steroids in stored urine samples*", Steroids (accepted for publication)

² Mean value of observations from different laboratories: 1.024 – in one case, specific gravity was measured at 1,016.

³ Mean value: 2.0 ng/mL (corrected_{1,020}: 1.6 ng/mL).

The stability test is conducted as follows:

To a test tube containing 4 to 5 µg of deuterated androsterone and etiocholanolone (-d₄ or -d₅), after careful evaporation of the solvent to dryness, is added 0.5 mL of the urine sample. The incubation is carried out at 37°C for approximately 15 hours. The free and hydrolysed glucuroconjugated steroids are isolated, derivatized and analysed preferably by high sensitivity GC/MS in the SIM mode. Ions corresponding to the respective deuterated and non deuterated 19-NA and 19-NE are monitored. Concentrations are estimated by comparison to an appropriate reference material.

In an unstable urine sample, there will be formation of deuterated and non deuterated 19-NE and to a lesser extent 19-NA. Unstable findings are characterised by 20% or more increase of the estimated amount of 19-NE (combined glucuroconjugated and free) and the presence of deuterated 19-NE.

Interpretation of stability results:

1) Findings in urine samples in which there is 20 % or more increase of non deuterated 19-NE (and to a lesser extent formation of 19-NA) plus the formation of deuterated 19-NE and deuterated 19-NA shall not be reported as adverse analytical findings;

2) When there is less conversion following incubation than described in 1), a GC/C/IRMS analysis of the norsteroids shall be conducted⁴. Confirmation of the 19-NA peak identity shall be conducted by GC/MS analysis under the same chromatographic conditions.

- If the GC/C/IRMS analysis reveals an exogenous source of 19-NA, the finding shall be reported as an adverse analytical finding.
- When the results of such an analysis conclusively exclude an exogenous origin of 19-NA, the finding shall not be reported. WADA Science Department shall be notified of all such results.
- When such analysis is not conclusive, the finding shall not be reported. WADA Science Department shall be notified of such inconclusive findings.

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⁴ Hebestreit, M., Flenker, U., Fußhöller, Geyer, H., Güntner, U., Mareck, U., Schänzer, W.: *Determination of the origin of urinary norandrosterone traces by GC/C/IRMS*. Publication in preparation.