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Quality control in steroid profiling

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Abstract

A simple and easy quality control method for urinary steroid profiling in doping control analysis is presented. An aliquot of the certified reference material (NMIA MX005) is analyzed with each batch of urine samples and the calculated values for the testosterone/epitestosterone (T/E) ratio and the concentrations of testosterone (T), epitestosterone (E), androsterone (A), etiocholanolone (Etio), 5α-androstane-3α,17β-diol (5αAdiol) and 5β-androstane-3α,17β-diol (5βAdiol) are monitored by means of quality control cards over time. The quality control cards consider different limits, as confidence intervals of the analysis certificate, the laboratory uncertainty estimated during the method validation or the expanded measurement uncertainty based on the World Anti-Doping Agency (WADA) technical document TD2014EAAS. When exceeding the uncertainty limits, corrective actions have to be performed.

Introduction

Since 1 January 2014 the reporting of the steroid profile from the Initial Testing Procedure for doping control urine samples is mandatory. The laboratory has to report via WADA´s Anti-Doping Administration and Management System (ADAMS) the testosterone/epitestosterone (T/E) ratio and the concentrations of testosterone (T), epitestosterone (E), androsterone (A), etiocholanolone (Etio), 5α-androstane-3α,17β-diol (5αAdiol) and 5β-androstane-3α,17β-diol (5βAdiol).
Regarding the fact, that all steroid profiles are followed up by the Athlete Biological Passport approach (Adaptive Model), an accurate measurement of the steroid profile parameter is of utmost importance.

Experimental

The quality control method is based on the use of the certified reference material (NMIA MX005, Steroid Metabolites in Freeze-Dried Human Urine, National Measurement Institute, Australia).
Aliquots of the reconstituted reference urine are prepared according to the common procedure for anabolic androgenic steroids [1,2] and analyzed with each batch of urine samples until exhaustion.

Results and Discussion

Quality control cards considering the following limits are established (Table 1):
a) Confidence levels of the certificate of analysis (Ref ± $U_{95\%\ Ref}$)
b) Expanded measurement Lab uncertainty - as estimated during method validation of the initial testing procedure by the laboratory (Ref ± $U_{95\%(Lab),\ k=2}$)
c) Expanded measurement uncertainty based on TD2014 EAAS (Ref ± $U_{95\%(TD);k=2}$) [3]

 ISBN 978-3-86884-040-7

In Figure 1 the T/E quality control card is presented. This principle is applicable to the other target steroids listed in Table 1. No significant differences in the quantitation of the steroid profile parameters as a function of the instrument were determined. When exceeding the inhouse uncertainty limits (blue line), corrective actions have to be performed and/or a recalculation of $U_{95\% (Lab)}$.
An out of control situation occurs when the technical document limits are exceeded (red line) for one or more parameters. In this case the steroid profile results are not valid and cannot be reported.Possible corrective actions are: preparation of fresh calibrators, new calibration, re-analysis of samples, instrument maintenance.

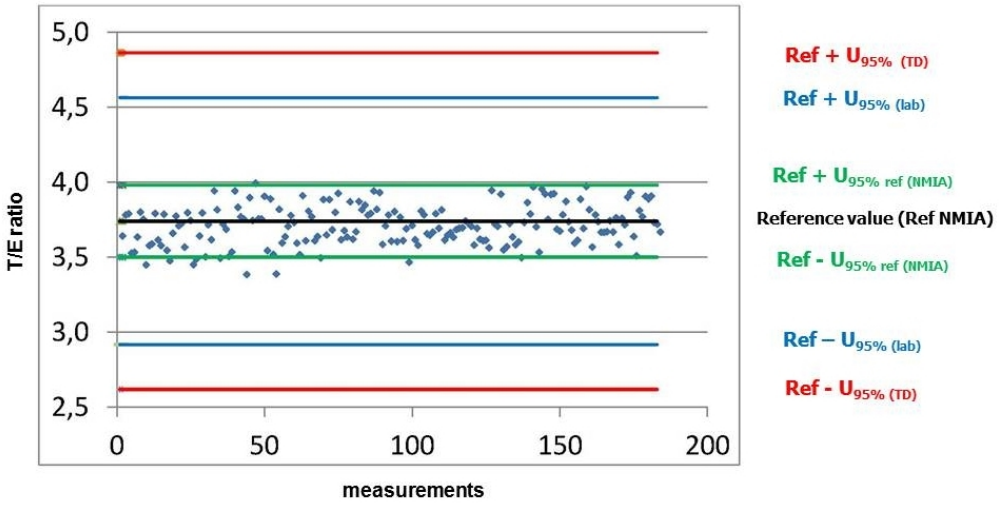

Table1: Basis for quality control card limits

Steroid	Confidence level of 95% (Ref ± U_{95ref}) Certificate of analysis NMIA MX005	$u_{c\ Max}$ (%) TD2014EAAS	$U_{95\% (TD)}$ TD2013DL
A	1187 ± 39 ng/mL	20	40
Etio	1293 ± 44 ng/mL	20	40
5αAdiol	7.27 ± 0.52 ng/mL	25	50
5βAdiol	21.1 ± 1.6 ng/mL	25	50
T	40.3 ± 1.7 ng/mL	20	40
E	10.76 ± 0.60 ng/mL	20	40
T/E	3.74 ± 0.24	15	30

$U_{c\ Max}$ (%): relative combined standard uncertainty (refer to TD2014EAAS)

$U_{95\% (TD)} = u_c(\%) \times k$, with k=2 Expanded measurement uncertainty (refer to TD2013DL v2)

Figure 1: T/E quality control card

Conclusions

This simple quality control method, based on the analysis of a certified urine included in each batch of urine samples provides an easy possibility to readily detect trends and out of control situations in steroid profile analysis.

References

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[2] Geyer H, Schänzer W, Mareck-Engelke U, Nolteernsting E, Opfermann G (1997) Screening Procedure for Anabolic Steroids – the Control of the Hydrolysis with Deuterated Androsterone Glucuronide and Studies with Direct Hydrolysis. In: Schänzer W, Geyer H, Gotzmann A, Mareck U (eds) *Recent Advances in Doping Analysis (5),* Köln, p 99-101
[3] WADA Technical Document TD2014EAAS, Version Number 1.0, Effective Date 1 January 2014

Acknowledgements

The authors wish to thank the Federal Ministry of the Interior of the Federal Republic of Germany and the Manfred Donike Institute (MDI) for support.