"Production and Certification of a Replacement Certified Reference Material for Human Urinary Steroids to ensure Quality of Longitudinal Profiling Data"

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Project Overview

The aim of the project is to produce a replacement freeze-dried human urine matrix certified reference material for the six steroids specified in the WADA technical document TD2014EAAS as markers for the urinary steroid profile. The material will be certified to a high level of accuracy with low uncertainties for the mass fraction and concentration of the steroid markers and testosterone to epitestosterone (T/E) ratio. The certified values will be assigned using isotope-dilution mass spectrometry and will be traceable to the International System of Units (SI). This material is intended to replace the stock of certified reference material NMIA MX005 which will be depleted over the next two years. MX005 has been used by WADA laboratories since its release in 2009 to demonstrate comparability of measurements now used for the Athlete Biological Passport (ABP). The ABP benefits all athletes by improving detection of doping and benefits individuals with unusual profiles by reducing unjustified scrutiny. The success of the ABP relies on results of analysis for all samples from an individual athlete being comparable irrespective of the WADA laboratory that performed the analysis. Comparability can be demonstrated by the appropriate use of a CRM specifically designed for the purpose.

Results and Conclusions:

A new freeze-dried human urine matrix certified reference material (CRM) has been produced to assist laboratories in longitudinal profiling measurements used for the Athlete Biological Passport and in the detection of testosterone abuse. Approximately 2400 units of the CRM (designated NMIA MX017) were produced and are now available to WADA accredited laboratories. The material is provided with SI traceable reference values for the mass fractions and mass concentrations of the glucuronides of six steroids specified in the WADA technical document TD2018EAAS as markers for the urinary steroid profile: testosterone (T), epitestosterone (E), androsterone, etiocholanolone, 5α-androstane-3α,17β-diol and 5β-androstane-3α17β-diol.

The CRM was produced using pooled urine from six healthy individuals that had been fortified with testosterone glucuronide to provide a T/E mass ratio of 4. The mass fractions and concentrations of the target steroid glucuronides in the reconstituted urine were determined using the primary ratio analytical method of exact matching double isotope dilution mass spectrometry (IDMS) following extensive sample clean-up. The analytical procedure developed and

optimised for this CRM employed a heart-cutting two-dimensional HPLC purification of the target analytes and quantification using five characteristic molecular fragments in tandem mass spectrometry or high-resolution mass spectrometry coupled with two different GC columns.

Estimates of the uncertainty in the certified values are based on investigation into all sources of uncertainty in inputs to the measurement equation and on studies of the homogeneity and stability of the mass fractions of the six steroid metabolites in the freeze-dried urine material during long-term storage, transport and use. Mass fraction and concentration reference values in the Certificate of Analysis for the CRM have expanded uncertainties (at the 95% level of confidence) below 5%. This is the same or better than previous similar CRMs, ensuring that NMIA MX017 will be a suitable replacement in controlling the quality of longitudinal profiling data.