

PROJECT REVIEW

“Production of Testosterone/Epitestosterone (T/E) Certified Reference Materials in Urine and Solution”

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Violations for the steroid testosterone are typically the second most common (after Cannabis) of all banned substances. To detect testosterone abuse an initial screening of athletes' urine occurs and the testosterone/epitestosterone ratio is measured. Enhanced levels of testosterone with respect to its very close analogue epitestosterone are indicative of steroid abuse. The new 2005 WADA Prohibited Substances List took effect on January 2005 and changed the level of the testosterone/epitestosterone ratio at which further investigation for potential testosterone abuse must be conducted from 6/1 to the lower level of 4/1.

The measurement of T/E ratios is technically not a trivial issue and his reduction in ratio makes it even more essential that WADA-accredited laboratories can accurately determine T/E ratios. A main aim of this project is the production and certification of such a urine matrix certified reference material (CRM) certified to a very accurate level for the concentrations of both testosterone and epitestosterone (and their ratio) will also be produced to provide laboratories with a suite of reference materials to ensure that as many parts of their analytical process as possible can be controlled.

These certified reference materials will be produced by Australia's National Measurement Institute (NMI) using techniques which are considered to be primary methods to ensure the highest accuracy and lowest uncertainty. Once they are produced these reference materials can be used by all WADA-accredited laboratories to test their specific methods and ensure accurate and internationally comparable results are produced. These materials will provide an unequivocal international benchmark for all measurements of these steroids around the world.

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Results and Conclusions

The preparation and characterisation of two matrix reference materials to underpin the accuracy of measurements of testosterone to epitestosterone (T/E) ratios has been successfully completed. A methanol solution of testosterone and epitestosterone was prepared as reference material NMIA MX006 and distributed into 2386 glass ampoules, each containing 1 mL. Composite human urine containing natural levels of epitestosterone glucuronide and testosterone glucuronide, the key excreted metabolites of testosterone and epitestosterone, was fortified with additional testosterone glucuronide to an approximate T/E ratio of 4. This material (designated NMIA MX005) was distributed into 1282 bottles in 20 mL aliquots and freeze-dried to maximise stability in storage.

During the first two years of the project, high accuracy methods of analysis for testosterone, epitestosterone and their glucuronides were developed, optimised and validated. Pilot batches of the two proposed reference materials were produced to demonstrate the feasibility of preparing homogenous and stable products with the required property values. This enabled successful preparation of bulk quantities of the candidate certified reference materials (CRM), which were then apportioned into individual units. These were then extensively evaluated to provide initial reference values and associated uncertainties during the second year of the project. Assessment of homogeneity and stability of the CRMs during storage, transport and use was performed and alternate high accuracy methods of analysis developed to investigate possible measurement biases.

Tasks performed during the third and final year of the project involved completing the estimates of the uncertainty associated with the reference values for the measurands to be certified. These measurands are the mass fractions and concentrations of testosterone and epitestosterone in the methanol solution (NMIA MX006), the mass fractions and concentrations of the glucuronide conjugates of testosterone (as testosterone) and epitestosterone (as epitestosterone) following reconstitution of the freeze dried urine (NMIA MX005), and the T/E ratios in both materials.

The long term stability trials on both reference materials at their normal storage temperature (-20°C) continued and no significant changes were observed in the measurands.