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Following steroid profiles outside the WADA management system (ADAMS)

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Abstract

In January 2014, the World Anti-Doping Agency (WADA) implemented the steroidal module of the athlete's biological passport. The evaluation of the individual steroid profile (normal, atypical, abnormal) is available to the athlete passport management unit (APMU) once the results are downloaded in ADAMS. The atypical and abnormal profiles are flagged to the AMPU and a request for confirmation is sent to the laboratory having performed the analysis.

Some major sport organizations are not using ADAMS but have requested longitudinal individual steroid profiling from our laboratory. In order to do so, we have developed a SQL Oracle database linking the athlete identification key provided by the sport, the bottle code and the test results. The profiles acquired during the initial GC-MS/MS test are extracted and compiled on a spreadsheet which can be accessed for evaluation. With this setup, we were able to monitor in 2013 approximately 1,400 individual male athlete's longitudinal profiles containing 1 to 10 tests each. This tool was found to be particularly useful for the laboratory, enabling a rapid selection of the samples requiring an IRMS analysis, which is currently not possible under ADAMS. In this paper, we present the database with a brief comparison with what is available from ADAMS to an APMU; examples of longitudinal studies are shown including examples of the impact of ethanol and the administration of a testosterone gel on real athletes' steroid profiles.

Introduction

The T/E value is the main marker of the administration of testosterone [1]; it is measured from hydrolyzed glucuronides during the initial testing of athletes' urine samples. The GC-C-IRMS confirms the synthetic or endogenous origin of urinary metabolites [2]. In order to efficiently program the IRMS analysis, considering the strong inter-individual differences in the excretion of the glucuronides of testosterone and epitestosterone, the individual profiling works best [3-5].

In January 2014, WADA implemented the steroid module of the athlete passport; through ADAMS, a notification is sent to the laboratory requesting a confirmation.

We have proposed to sport organizations not utilising ADAMS, to follow the steroid profiles internally. The identity of the athletes remains unknown and our review directs the appropriate course of actions.

Experimental

<u>Analysis of urine samples</u>

Samples are analysed with the initial testing procedure implemented and validated years ago. Typically, the free and conjugated steroids are isolated by solid phase extraction (Sep Pack C18 cartridges) followed by the enzymatic hydrolysis in phosphate buffer (pH 6.9) with purified β-glucuronidase from E. coli (type IX, lyophilised, 1,200 units) and liquid-liquid extraction diethyl ether at pH 9 (carbonate buffer). The TMS-derivatives are obtained and analysed on an Agilent GC-triple Quadrupole (GC-TQ) model 7000A (GC model 7890A) operating under the Mass Hunter software.

Steroid profile:

The T/E value is the area ratio of the testosterone and epitestosterone peaks (ion-transition 432 to 209), concentration of DHEA (432-303), 5α- and 5β-androstanediol (346-256), androsterone, etiocholanolone (434-329) are measured as well. Three-point calibration standards, quality control samples including all the analytes are injected with each lot. The uncertainty of each measure was estimated in function of the concentration.

Information provided by the Sport Organization:

The collection dates, sample codes, specific gravity values and identification codes are contained in CSV files located on a secured FTP server. The identification code (Subject Key, ADAMS or SIMON identification) is used to anonymously link sample codes to individual athletes.

Matching information and initial test results:

A specialized download tool (Cogilab, Québec) retrieves the CSV event files and stores the information on the laboratory Oracle SQL server. The LIMS (Cogilab, Québec) retrieves this information, matches it with the affixed laboratory code and sample information (gender, specific gravity, sport, organization) and sends this data to the GC-MS/MS. Quantitation results are exported back. The Steroid Profile Review Tool queries the SQL server, generates and handles a profile review worksheet by adding current test results to existing steroid profile data. The interactive process is summarized at Figure 1.
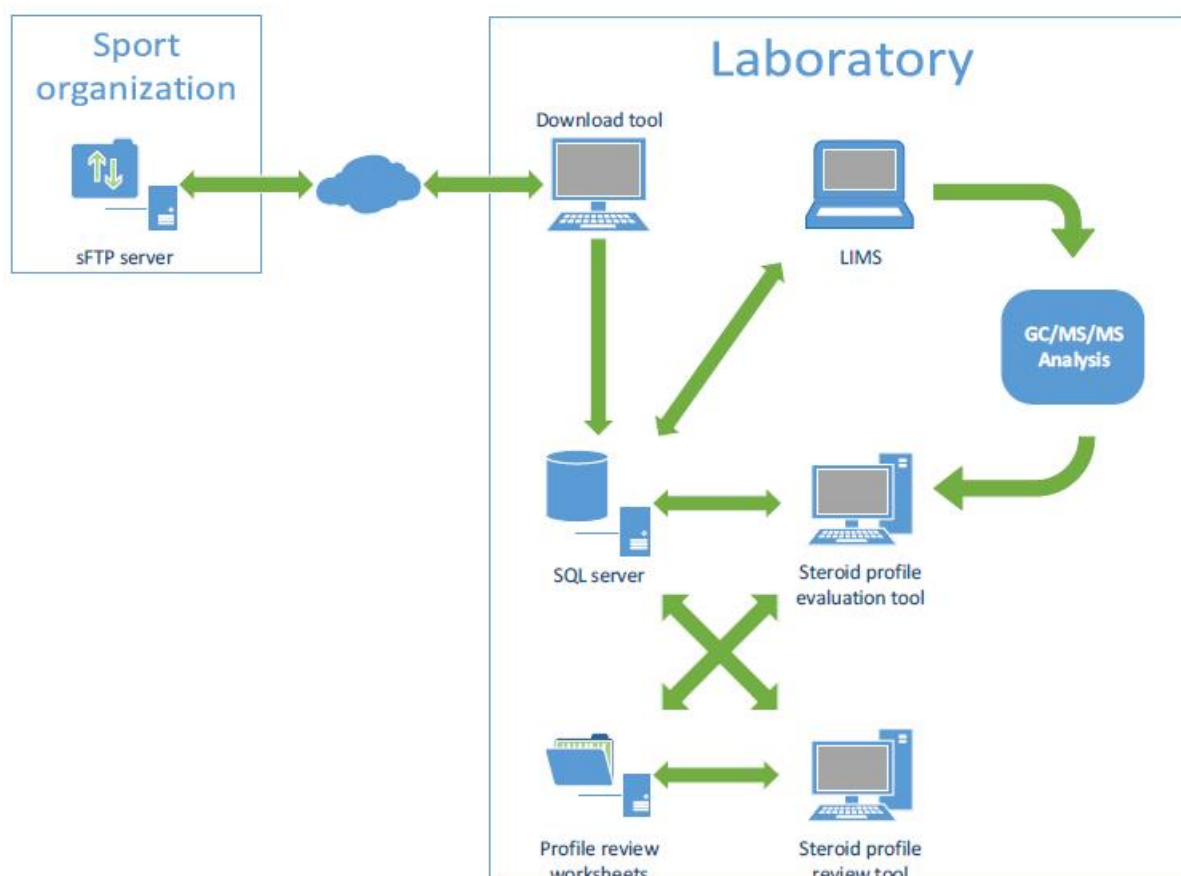

Figure 1: Scheme summarising the exchange of external (Sport organization) and internally generated information permitting the generation of individual steroid profiles.

Results and Discussion

We have selected a population of 1,238 male athletes' profiles each containing 2 to 10 tests. The mean individual T/E values ranged from 0.02 to 7.7.

The graph in Figure 2 is a plot of the mean individual T/E values and their coefficient of variation (%). Most of individual T/E values vary by less than 30% (mean c.v.: 16.4% ± 12.2%). The wider variation was observed in the group of 148 T/E values ranging from 0.02 to 0.1 (mean c.v.: 22.4 % ± 19.5%) with low concentrations of testosterone at 4.0 ng/mL ± 1.7 ng/mL (means, adjusted to a specific gravity value of 1.020).

As shown by the graph of Figure 2, the majority of findings with the higher variations could be explained by ethanol (free or conjugated) present in 31 cases (green points), microbial degradation in 1 (red) and the administration of a testosterone gel (yellow). For the latter, the T/E values ranged from 0.12 to 0.96 (mean T/E: 0.5 ± 0.4), the concentration of testosterone from 0.3 to 5.7 ng/mL (adjusted at specific gravity value of 1.020). Although the concentrations of testosterone were in the lower 1% percentile range, the results of the GC-C-IRMS were clearly supportive of the exogenous origin.

The influence of ethanol ingestion on the steroid profile is known since the 1990s [6]. An illustration is provided in Figure 3, a worksheet produced by the evaluation tool for an athlete's normal profile from which an outlier T/E value at 5.4 was excluded since related to the presence of ethanol (ethyl-G: 157 µg/mL, ethyl-S: 59 µg/mL) as confirmed by the results of the GC-C-IRMS analysis.
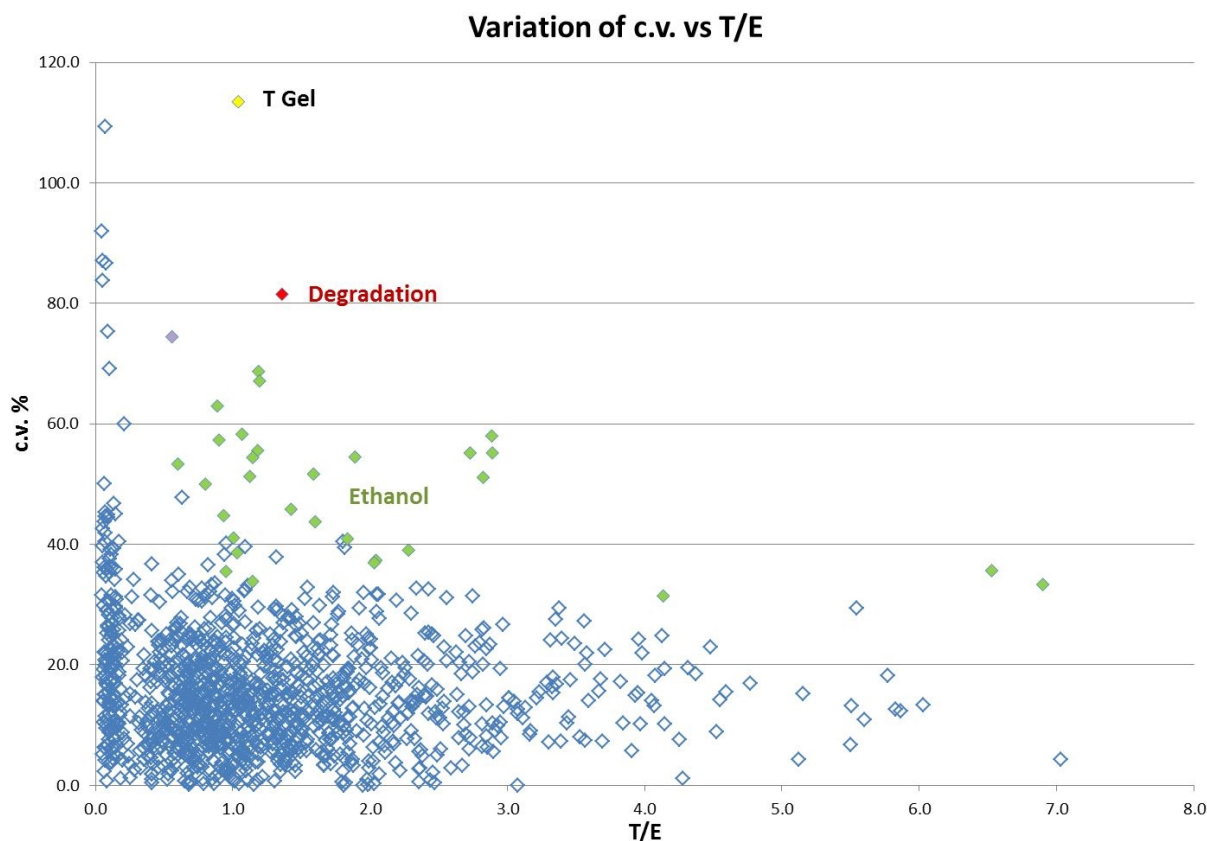

Figure 2: Coefficients of variation vs. mean T/E values in 1,238 individual male athletes' profiles containing more than 1 test result: high values correlated with administration of Testosterone gel (yellow), microbial degradation (red) and presence of ethanol (green).

Figure 3: Worksheet produced by the evaluation tool for an athlete's profile influenced by ethanol (outlier T/E value at 5.4).

Conclusions

For organizations employing a single laboratory and handling results outside ADAMS, this tool enables the rapid review of individual athlete's steroid profiles, which is the best way to lower the number of false negatives and unnecessary IRMS analyses. The decision to verify the validity of the initial test results (typographic mistakes, wrong bottle, integration or peak identification, microbial degradation, ethanol or other factor altering the profile [7]) or to proceed with the confirmation, is with the laboratory. The results acquired years before can be included. The conclusion reached by the laboratory can be annotated promptly, outliers caused by ethanol, or microbial degradation excluded from the subject-based normal values.

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