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Implementation of steroidal passport: Experiences of Indian laboratory

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ABSTRACT

The Athlete Biological Passport (ABP) is an indirect approach which provides a complementary and more sophisticated strategy to traditional analytical testing in an effort to scientifically gather evidence of possible doping in sport. The ABP is one tool in a kit of intelligent anti-doping practices meant to deter and detect the use of prohibited substances in sport. In 2013, the WADA Athlete Biological Passport Guidelines introduced a second module, the Steroidal Module, which became operational since January 1, 2014. The Steroidal Module monitors an athlete's steroidal variables over time that may be indicative of steroid abuse. This paper summarized the details of samples requested for confirmation on GC/C/IRMS in year 2015 & 2016 to understand the pattern of generation of Atypical Passport Finding Confirmation Procedure Request (ATPF-CPR). Interestingly, out of total 26 cases of ATPF-CPR received by NDTL, three samples with normal steroid profile showed exogenous origin of endogenous steroids on GC/C/IRMS analysis, which proves the effectiveness of Steroidal Module. In this context, monitoring of steroid passport through steroidal module represents the new paradigm in detection of exogenous origin of endogenous steroids.

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1. Introduction

The administration of Endogenous Anabolic Androgenic Steroids (EAAS) can alter levels of one or more of the Markers and/or ratios of the urinary "steroid profile".[1–3] World Anti-Doping Agency (WADA) introduces Steroidal Biological Passport Module in year 2013 which was applicable since January 1, 2014. The Steroidal Module monitors an athlete's steroidal variables over time that may be indicative of endogenous steroid abuse. This Adaptive Model based on Bayesian inference[4] replaced the 'population reference' approach with an 'intra-individual' approach, allowing evaluation of steroid profile in more investigative manner. Steroidal Module generates two type of ADAMS notification through adaptive module. One is Suspicious Steroid Profile-Confirmation Procedure Request (SSP-CPR) which is based on the criteria laid down in section 3.0 of WADA TD2016EAAS.[5] Second type of notification is Atypical Passport Findings-confirmation Procedure Request (ATPF-CPR). The Adaptive Model is an algorithm that calculates whether the result, or results over the time in the case of a longitudinal profile, is likely the result of a normal physiological condition. An Atypical Passport Finding (ATPF) is generated in ADAMS if the athlete's Testosterone/Epitestosterone (T/E) ratio is out of the individual range generated by the Adaptive Model to a specificity of 99%. An Atypical Passport Finding (ATPF) requires further investigation.[6]

The present work summarizes the experiences of India Lab with ATPF-CPR & SSP-CPR handled during year 2015 & 2016. The effectiveness of the present Steroidal Module as a part of strict Doping Control Program is also evaluated in Indian Scenario.

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2. Materials and Methods

2.1. Chemicals and reference standards

Reference standards of endogenous steroids and deuterated internal standards were procured from Sigma-Aldrich (USA) and National Measurement Institute (Australia). C-18 sample preparation cartridges were procured from RFCL Ltd, β-glucuronidase enzyme (E. coli) was from Roche Diagnostics (USA). All other solvents and chemicals were of high performance liquid chromatography (HPLC) grade and analytical grade respectively.

2.2. Sample preparation for steroid profiling

The sample preparation procedure was same as followed in routine analysis for steroids screening involving solid phase clean up, enzymatic hydrolysis, solvent extraction and derivatization, followed by Gas Chromatography-Mass Spectrometry (GC-MSD & GC-MS/MS) analysis was employed.[7]

2.3. Gas Chromatography/Combustion/Isotope Ratio Mass Spectrometry (GC/C/IRMS) analysis

Upon receipt of SSP-CPR(s) or ATPF-CPR(s) after consultation with relevant Testing authority, Confirmation procedure was initiated employing the duly validated method for GC/C/IRMS analysis which was used in routine doping analysis at NDTL. Delta ^{13}C values of metabolites of testosterone: Androsterone (Andro), etiocholanolone (Etio), 5α-androsterone-3α-17βdiol (5α-Adiol) and 5β-androsterone-3α-17βdiol (5β-Adiol) along with Testosterone (T) and Epitestosterone (E) were measured. 11 keto-etiocholanolone (11 keto) and Pregnanediol (PD) were used as endogenous reference compound (ERC).

3. Results and Discussion

In year 2015 & 2016, Indian Lab received total 101 SSP-CPR(s) and 33 ATPF-CPR(s). Percentage positive in both the years for SSP-CPR were almost similar. But in case of ATPF-CPR, only in year 2016, three samples out of sixteen samples showed exogenous origin of endogenous steroids. However, the steroidal parameters were within the normal range of steroid profile. Two of the ATPF-CPR showed inconclusive results as the concentration of various steroid parameters were below LOD of the assay, though the T/E ratio in both the samples was in the range of 3.0 - 4.0.

3.1. Suspicious steroid profile-confirmation procedure request (SSP-CPR)

Detailed analysis of SSP-CPRs received in year 2015 & 2016, revealed that out of 101 samples with suspicious steroid profile, 24(23.8%) samples showed exogenous origin of endogenous steroids. Out of 24 exogenous cases, 23(96%) belongs to the category of T/E ratio more than 6 (Table 2 &Table 3), only single sample showed exogenous origin of endogenous steroid on the basis of high concentration of Androsterone. SSP-CPR(s) received for various steroid parameter other than T/E ratio, such as A/T<20, 5a-Adiol/5b-Adiol> 2.4, 5a-Adiol/E, 5a-Adiol>250/150ng/ml, was concluded as endogenous.

Details provided inTable 3 clearly indicated that only 23 samples out of 51 (45%) having T/E ratio above 6, showed exogenous origin of testosterone or its prohormone

A further in-depth analysis of steroid profile on the basis of steroidal module/ longitudinal profile, is required to avoid unnecessary confirmation analysis on GC/C/IRMS.

3.2. Atypical passport findings-confirmation procedure request(s)

In general an Atypical Passport Finding (ATPF) is generated in ADAMS if the athlete's Testosterone/Epitestosterone (T/E) ratio is out of the individual range generated by the Adaptive Model. In year 2015 & 2016, total 33 ATPF-CPRs were received and tested by Indian Laboratory. In 2015 all the ATPFs showed endogenous findings on GC/C/IRMS, while in year 2016, 18.75% samples showed exogenous origin of endogenous steroids and 12.5% were reported as inconclusive due to low concentration of target compound in urine sampleTable 4. Reanalysis was performed by grouping T/E ratio in three different ranges. All the sample with T/E ratio below 1 resulted in an endogenous finding.

The three positive cases of ATPF-CPR received at NDTL substantiate the effectiveness of the steroidal module in Indian Scenario. In case the longitudinal steroid profile of these sample were not monitored by Testing authority/Athlete Passport Management Units (APMU), the dope tainted athletes could have surpassed the Anti-doping System. T/E ratio of exogenous ATPF-CPR samples are given in$.

4. Conclusion

Monitoring of steroid profile through steroidal module represents the new paradigm in detection of exogenous origin of endogenous steroids.

More effective implementation and strict monitoring of steroid passport through APMU by various Testing Authorities/NADOs is a must for true fight against doping in sports.

5. Source of Funding

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6. Conflict of Interest

None.

Table 1: Details of SSP-CPR and ATPF-CPR analysis in the year 2015 & 2016

Notifications Type	Year	Total No. of Samples	Results Reported		
			Exogenous	Endogenous	Inconclusive
SSP	2015	53	14 (26.4%)	39 (73.6%)	0
	2016	48	10 (20.8%)	38 (79.2%)	0
ATPF	2015	17	0	17 (100%)	0
	2016	16	3 (18.8%)	11 (68.7%)	2 (12.5%)

Table 2: Detailed analysis of SSP-CPR received in year 2015 & 2016

SSP-CPR Parameters	Year	Total No. Of Samples	Result Reported	
			Exogenous	Endogenous
T/E >4	2015	52	13 (25%)	39 (75%)
	2016	35	10 (28.6%)	25 (71.4%)
A/T <20	2015	0	0	0
	2016	3	0	3 (100%)
A/T & 5a/E	2015	0	0	0
	2016	1	0	1(100%)
5a/5b >2.4	2015	0	0	0
	2016	1	0	1 (100%)
5a >250/150 & 5a/E	2015	0	0	0
	2016	4	0	4 (100%)
5a>250/150	2015	0	0	0
	2016	3	0	3 (100%)
5a/5b & 5a/E	2015	0	0	0
	2016	1	0	1 (100%)
Profile (high Andro)	2015	1	1 (100%)	0
	2016	0	0	0

Table 3: Detailed analysis of SSP-CPR on the basis of range of T/E ratio

Range of T/E	Year	Total No. of Samples	Result Reported	
			Exogenous	Endogenous
T/E<4	2015	1	1 (100%)	0
	2016	15	0	15 (100%)
T/E (4-6)	2015	21	0	21 (100%)
	2016	13	0	13 (100%)
T/E (6-8)	2015	15	2 (13.3%)	13 (86.7%)
	2016	7	3 (42.9%)	4 (57.1%)
T/E (8-10)	2015	6	2 (33.3%)	4 (66.7%)
	2016	4	1 (25%)	3 (75%)
T/E>10	2015	10	9 (90%)	1 (10%)
	2016	9	6 (66.7%)	3 (33.3%)

Table 4: Detailed analysis of ATPF-CPR samples

Range of T/E	Year	Total No. of samples	Result Reported			Range of T (ng/ml)	Range of E (ng/ml)
			Exogenous	Endogenous	Inconclusive		
T/E<1	2015	13	0	13 (100%)	0	0.3 - 26.7	6.9 - 96
	2016	6	0	6 (100%)	0	0.5 - 21.3	9.3 - 107.6
T/E (1-2)	2015	2	0	2 (100%)	0	35.4 - 38.3	21.1 - 24
	2016	6	2 (33.3%)	4 (66.7%)	0	2.0 -28.2	1.45 - 26.2
T/E (2-4)	2015	2	0	2 (100%)	0	36.3 - 48.6	11.7 - 18.7
	2016	4	1 (25%)	1 (25%)	2 (50%)	3.7 - 76.2	1.13 -20.6

Table 5: T/E ratio of positive cases of ATPF-CPR

T/E ratio	GC/C/IRMS Analysis	Remarks	Conc. of Testo (ng/ml)
1.65	Exogenous		2.4
1.67	Exogenous		5.3
3.71	Exogenous	AAF for Stanozolol also	76.2

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