The Athlete Biological Passport: a novel tool for the fight against doping in continuous evolution

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Principles of Athlete Biological Passport

1: Individual

2: Longitudinal

3: Based on an adaptive model

4: Defines normal individual limits for biological parameters

5: Extended monitoring of doping practices

6: Defining testing strategies
1. Reference values are set adapted to the athlete on first point
2. New upper and lower limits are calculated for second point
3. This process continues with the next points
4. The more points you have, the more accurate is the model (min. 4)
5. If a value goes out of the limits, the passport is flagged!
Haematological Module

4 blood markers

7 parameters

Haemoglobin (HGB)
Reticulocytes percentage (RET%)
Haematocrit (HCT)
Red blood cell count (RBC)
Mean corpuscular volume (MCV)
Mean corpuscular haemoglobin (MCH)
Mean corpuscular haemoglobin concentration (MCHC)

EPO/ESA abuse

Blood Transfusion
Example: EPO/ESA abuse

Continuous, slight increase in Haemoglobin concentration

The OFF score amplifies the changes observed in Haemoglobin and Reticulocytes.

Abnormal increase in Reticulocytes (samples 3-7, "ON phase") followed by marked drop when EPO is withdrawn and Erythropoiesis is suppressed (samples 8-9, "OFF phase").

Biological passport: EPO/ESA abuse

The samples 2-10 were taken on a regular base over a period of ~8 weeks.
Example: Blood Transfusion

Haemoglobin concentration (g/l)

- Large variation in Haemoglobin concentration after blood withdrawal and reinfusion.

- The OFF score amplifies the changes observed in Haemoglobin and Reticulocytes.

- High Reticulocytes paired with low Haemoglobin concentration suggesting hyperproliferative condition after blood withdrawal (samples 4+5).
- Low Reticulocytes with high Hb indicating suppressed erythropoiesis after reinfusion of blood (samples 6+7).

Reticulocytes (%)

Biological passport: Blood Transfusion

- The samples 2-10 were taken on a regular base over a period of ~8 weeks.
Steroidal Module

5 urinary markers

6 parameters

Testosterone (T)
Epitestosterone (E)
Androsterone (A)
Etiocholanolone (Etio)
5α-androstan-3α,17β-diol (5αAdiol)
5β-androstan-3α,17β-diol (5bAdiol)

EAAS abuse
(testosterone)

Muscle mass increase recovery
Endogenous Steroids – Historical Background

**INJECTION**
- Nebid T undecanoate
- Sustanon T decanoate, T isocaproate, T phenylpropionate, T propionate
- Testoviron T enanthate
- Testovis T propionate
- Testoviron T enanthate
- Testovis T propionate

**TRANSDERMAL**
- Testopatch
- Androgel
- Testim
- Testogel
- Tostrex

**ORAL**
- Andriol T undecanoate

**Muscle Mass Increase**

**Recovery**

**TESTOSTERONE DOPING DETECTION**
- **80’s**
  - T/E > 6
  - CIO Medical Commission
- **1992**
  - T/E > 6
  - Physiological Pathological condition
- **End of 90’s**
  - T/E > 6
  - IRMS
- **2008**
  - T/E > 4
  - IRMS
C. Ayotte
Detecting the administration of endogenous anabolic androgenic steroids
Doping in Sports (2010), Springer

P. Anielski et al.
Epidemiological investigation of the UGT2B17 polymorphism in doping control urine samples and its correlation to T/E ratios.
**Phase II Metabolism**

Glucuronide moiety (G)

\[
R = \overset{\text{CO}_2\text{H}}{\overset{\text{OH}}{\overset{\text{Xn}}{\text{OH}}}} = \overset{\text{Gluconic acid}}{\overset{\text{Glucuronidase}}{\overset{\text{G}}{\text{O}}}}
\]

**Target Compounds of Steroidal Module**

- **Epitestosterone (E)**
- **Testosterone (T)**
- **Androsterone (A)**
- **Etiocholanolone (Etio)**
- **5α-androstane-3α,17β-diol (5αAdiol)**
- **5β-androstane-3α,17β-diol (5βAdiol)**

**Steroid Profile Analysis**

1. 2.5mL Urine → Hydrolysis (β-glucuronidase)
2. LLE (MTBE, 5mL) → Evaporation
3. Derivatization (MSTFA, 50µL, 20 min 60°C)
4. Injection (1µL)
5. GC-MS/MS Run Time 25 min
Real Case: T Administration

Oral T intake
T/E
A/T
[T], [A], [Etio], [Diols]

Transdermale T intake
T/E
5aAdiol/E
[T]
[E]

Influence of Ethanol
T/E
5aAdiol/E
A/T

Select# | DCF TA | Sample Code | Test Type | Date of Test | Valid | T [ng/mL] | E [ng/mL] | T/E | A/T [ng/mL] | A [ng/mL] | Eto [ng/mL] | A/Eto | 5a-diol [ng/mL] | 5b-diol [ng/mL] | 5a-d [ng/mL] | Specific Gravity
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
3233846 | OOC | 17-Sep-2017 | Valid | 25 31 | 0.82 | - | 54 | 1400 | 810 | 1.7 | 29 | 31 | 0.57 | 0.94 | 1.011
4080540 | OOC | 22-Mar-2017 | Invalid | < LOQ < LOQ | 0.00 | - | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | 1.8 | 1.8 | 1.011
4080540 | OOC | 23-Mar-2017 | Valid | 80 44 | 1.8 | 1.8 | 170 | 3000 | 8400 | 1.6 | 84 | 340 | 0.35 | 1.9 | 1.016
3991448 | OOC | 23-Aug-2016 | Valid | 55 49 | 1.1 | - | 38 | 2000 | 1700 | 1.2 | 31 | 110 | 0.46 | 1.0 | 1.024
3322224 | INC | 10-Mar-2016 | Valid | 27 29 | 0.95 | - | 120 | 3200 | 1100 | 2.9 | 44 | 53 | 0.82 | 1.3 | 1.017
3321667 | INC | 19-Feb-2016 | Valid | 28 39 | 0.73 | - | 110 | 3100 | 1100 | 2.9 | 53 | 67 | 0.79 | 1.4 | 1.015
3317762 | INC | 30-Sep-2015 | Valid | 43 49 | 0.93 | - | 44 | 1900 | 880 | 2.1 | 36 | 66 | 0.55 | 0.81 | 1.016
3320175 | INC | 25-Aug-2015 | Valid | 61 58 | 1.1 | - | 97 | 5900 | 2700 | 2.2 | 130 | 120 | 1.1 | 2.2 | 1.011

DOPING!
Analytical methods – hematological profiling

- The instruments are those used in clinical chemistry in health care
- Also the parameters are those targeted in hematological test (e.g. Hb, HCT, Ret)
- Highly automated processes use chemicals to dye and shape the cells
- The core element is a flow cell which exposes the cell populations with laser light
Analytical methods – steroid profiling

- The analysis is based on gas chromatographic (GC) separation and mass spectrometric (MS) detection of the target compounds in urine sample
- Sample preparation procedures highly harmonized within anti-doping laboratories
Confounding Factors

Factors that are capable of influencing either the measurement of the ABP markers or their interpretation

**Haematological Module**

**PHYSIOLOGICAL**
- Exercise
- Altitude
- Haemodilution (change of plasmatic volume)
- Seasonal Fluctuations

**EXTERNAL**
- Injuries
- Iron Supplement

**PATHOLOGICAL**
- Ulcers
- Gastrointestinal Diseases (Variations in [HGB])

**Steroidal Module**

**GENETIC**
- UGT2B17 enzyme deletion
- [T] (lower sensitivity)

**METABOLIC**
- “Conazols”
- 5a-reductase inhibitors
- Anabolic steroids
- Alcohol
- A/Etio
- 5aAdiol/5bAdiol
- Steroidogenesis inhibition

**EX-VIVO**
- Microbial contamination
- [5aAdiol] (5aAdiol/5bAdiol 5aAdiol/E)
A step forward...

**BLOOD MATRIX**

- Not easy to manipulate
- Reduced bacterial contamination
- Snapshot of athletes’ physiological condition
- Accurate pharmacokinetics information
- Trend in clinical analyses (evaluation/expertise)
- 1 Sample -> 2 ABP modules

**URINE DRAWBACKS**

- Confounding factors
  - ENDOGENOUS
    - UGT2B17 polymorphism
  - EXOGENOUS
    - Bacterial contamination
    - Ethanol consumption

- GC-MS Sensitivity
  - T patch & gel administration

- Few data in doping context
- Invasive sampling (ethics)
- Sample stability (48h)
- Transportation strategy
- Small sample volume (4mL)

T. Kuuranne et al.
*Confounding factors and genetic polymorphism in the evaluation of individual steroid profiling.*
F. Ponzetto et al.
Longitudinal monitoring of endogenous steroids in human serum by UHPLC-MS/MS as a tool to detect testosterone abuse in sports.

UGT2B17 enzyme

**ins/ins**
**ins/del**
**del/del**

**T intake**
**IAAF – Differences of Sex Development**

**Presence of one of the following DSDs**
- 5α reductase type 2 deficiency
- Partial Androgen Insensitivity Syndrome (PAIS)
- 17β-HSD3 deficiency
- Congenital Adrenal Hyperplasia (CAH)
- 3β-hydroxysteroid dehydrogenase deficiency
- Ovotesticular DSD
- Any other DSD involving gonadal steroidogenesis

**As a result:**
1. Blood T > 5nmol/L
2. Sufficient androgen sensitivity to have a material androgenising effect

**TO BE ELIGIBLE**

A) Must be recognized at law as female or intersex
B) Must reduce her blood T <5nmol/L for 6 months
C) Must maintain her blood T <5nmol/L

**Track Events**
- 400m
- 400m Hurdles
- 800m
- 1500m

**400m < Track Events < 1 Mile**
- Alone / Relay / Combined
Performance as a tool for intelligent testing

**PREDICTION**

S. Iljukov et al.
*Application of the Athlete’s Performance Passport for doping control: a case report.*

**LIVE MONITORING**

R. Phaiss
*Can power data in cycling contribute to an Athlete Performance Passport?*
SGS Conference – Maglingen 2018
Thank you for the attention!