



WADA / Science and Investigations Symposium - Istanbul

Beyond Analytical Results

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www.doping.chuv.ch



Unil
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Faculté de biologie
et de médecine

Where Science meets Investigations

- Already a long experience
- The forensic approach of anti-doping
- How DNA can help
- Beyond the passport
- The Prevalence of doping /Clean sport
- Pharmacokinetic / Re-analyses
- Where do we go?

Where Science meets Investigations

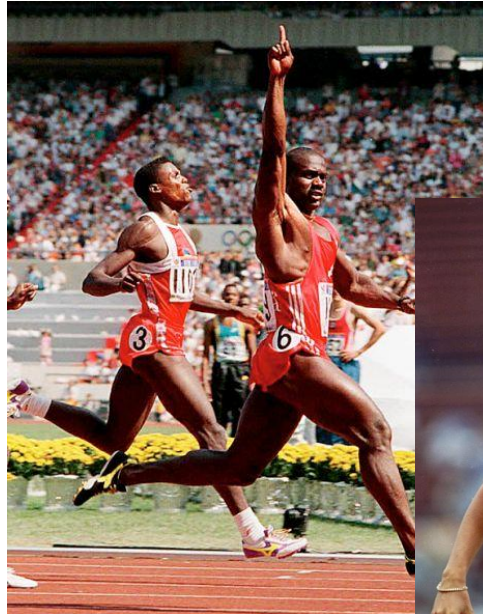
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Already a long experience

The Laboratory interacts with ADO



Prof. Donike
Cologne Laboratory



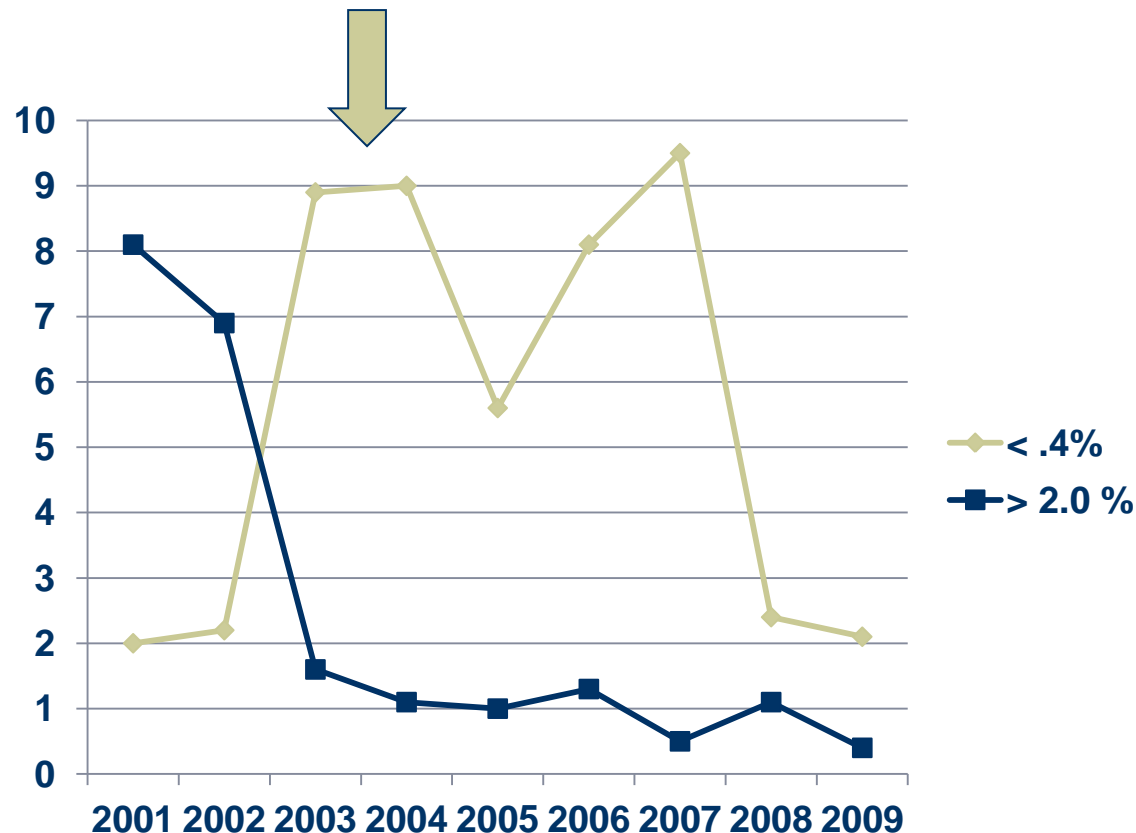
Prof. Ljungqvist
IAAF

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The forensic approach of anti-doping

2003-2004: After investigations on blood parameters in the population of professional cyclists, the prevalence of low reticulocytes showed an obvious re-appearance of blood transfusion in the field...



Zorzoli & Rossi, 2010, 2012

The forensic approach of anti-doping

After the Hamilton case, the Landis case and the Puerto affair, WADA promotes the forensic approach in the fight against doping.



The first step was the introduction of the Athlete Biological Passport based on a collaboration between WADA , UCI and the Lausanne Laboratory (LAD)



At this stage, the LAD set up a collaboration with the University Institute of Scientific Police & Criminal Sciences.
2 specialists have been hired to explore the Anti-doping intelligence in close collaboration with the Laboratory.

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How DNA can help

In 2007, medical equipment was found in a dustbin behind a team hotel during an international rowing competition (Rotsee, Switzerland). The LAD received the mandate by FISA to cooperate for an investigation.



More than 30 bottles of drugs and several syringes found in a dustbin.

No forbidden substances.

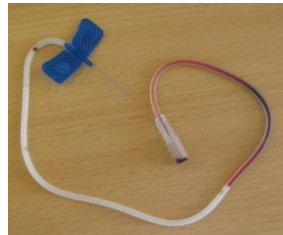
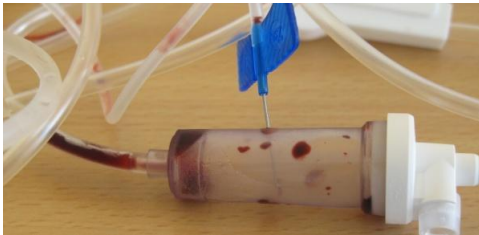
13 infusion systems.

Jan & al,

Forensic Sci Int. 2011;213:109-13.

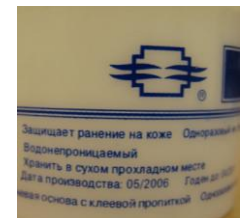
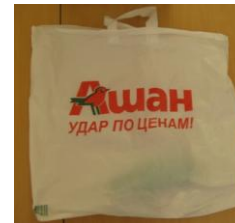
How DNA can help

DNA analyses on red residues from 10 infusion systems

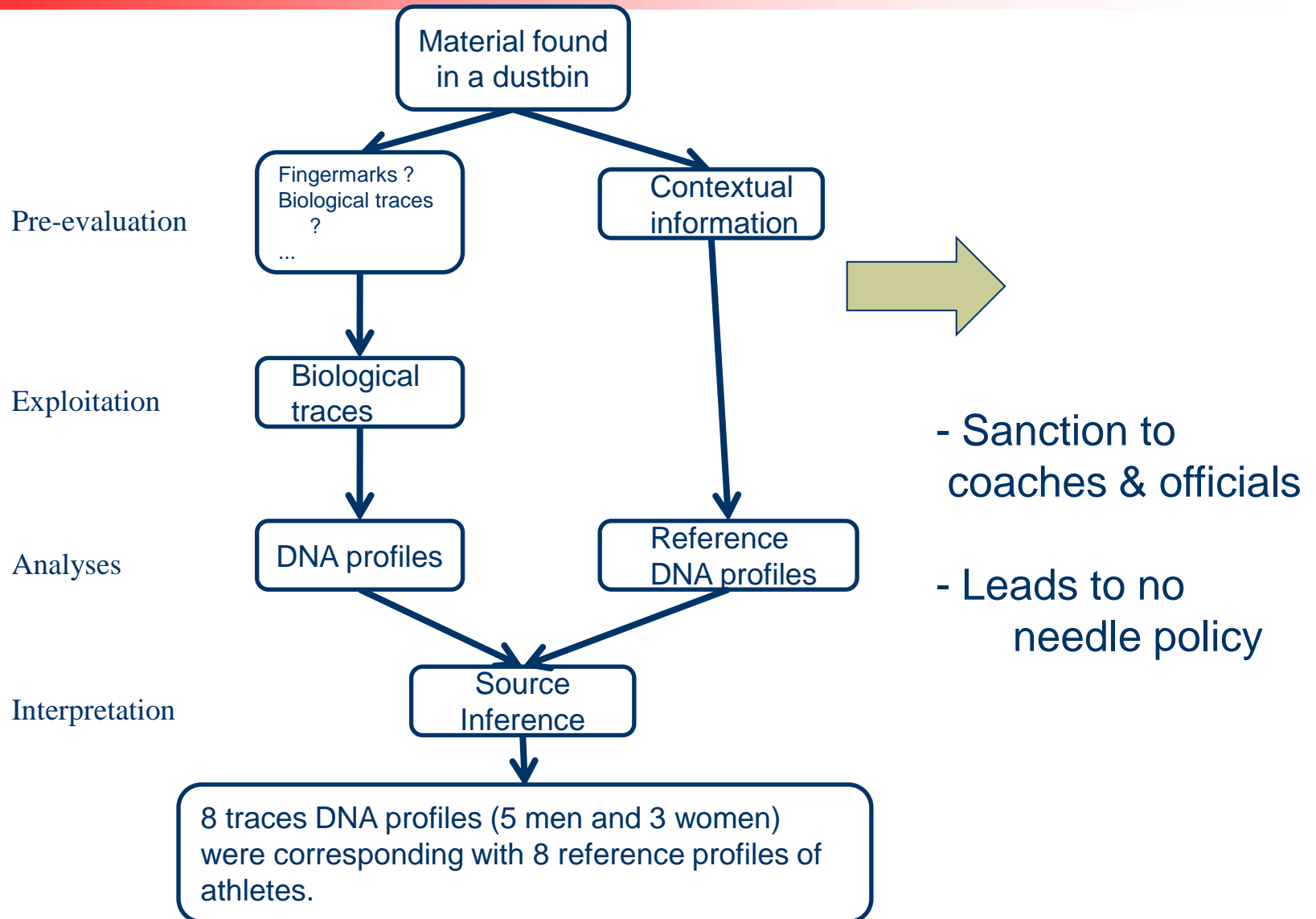


Circumstantial information

- Dustbin location
- Inscriptions on packaging



How DNA can help



How DNA can help

In 2007-2008, IAAF used DNA to control potential manipulation of the athlete's urine sample

Based on differences in steroid profiles, IAAF suspected that several athletes were not providing their own urine during out of competition controls.

DNA comparison between IC and OOC urine were performed.

Urines from 7 athletes were not corresponding, after comparison also with reference blood samples

They were charged because of urine substitution and have been suspended for a period of 2 years and 9 months.

CAS 2008/A/1718 to CAS 2008/A/1724

<http://www.tas-cas.org/d2wfiles/document/3767/5048/0/20091118165643673.pdf> (last visited May 2, 2010).

How DNA can help

Legitimacy and constraints of DNA tests in Anti-Doping

Implementation of DNA profiling is described in 2015 WADA code,

BUT :

1. Need to harmonize the profiling kits (loci/markers)
2. Compatible with data protection ?
3. DNA profiling doesn't provide information about suspicious result, but only on the identity of the donor.

To our point of view, there is a necessity to develop a standardized model of DNA profiling through a DNA testing policy with guidelines or technical document.

**Inclusion of DNA profiling in the Athlete's passport ?
Would be a progress...**

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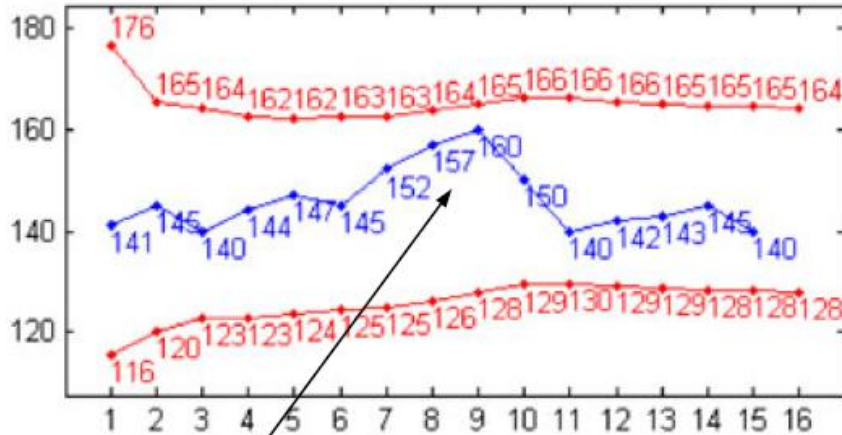
Beyond the passport

The passport, by the
individual and longitudinal
examination of the biological pattern from the athlete
is one of the best example of the link between
Science and Investigation

The technical application of the passport is using
a body of evidences
to assess the normality or abnormality of a biological profile,
then possibly to declare an adverse passport finding.

Beyond the passport

Haemoglobin concentration (g/l)

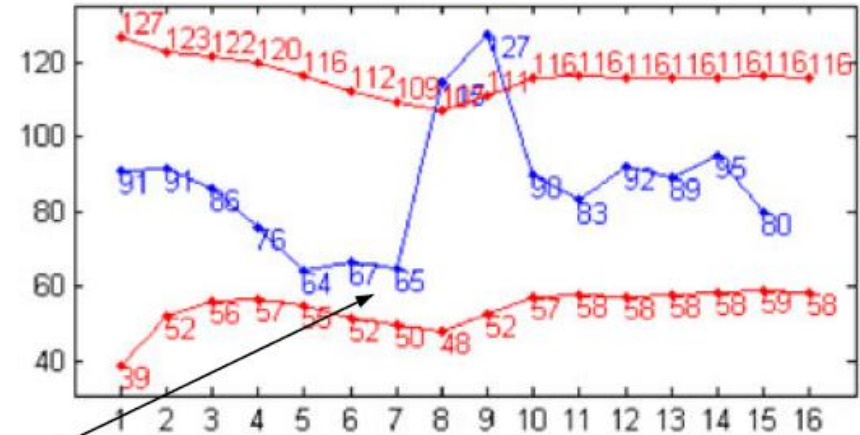


Continuous, slight increase in Haemoglobin concentration

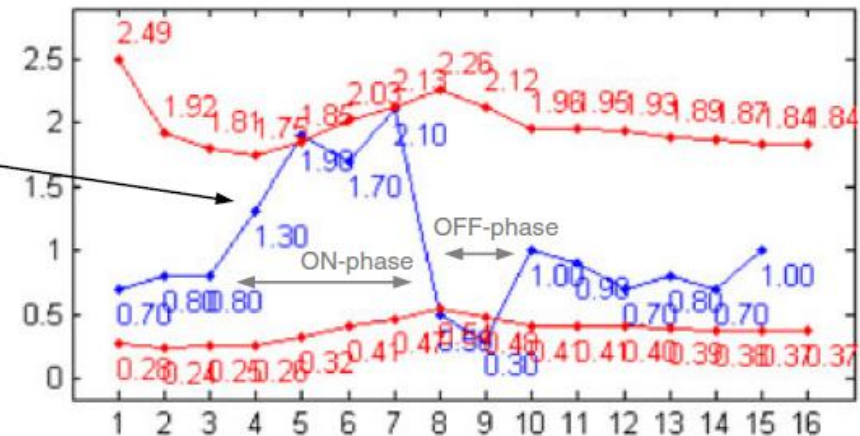
The OFF score amplifies the changes observed in Haemoglobin and Reticulocytes.

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“).

OFF Score



Reticulocytes (%)

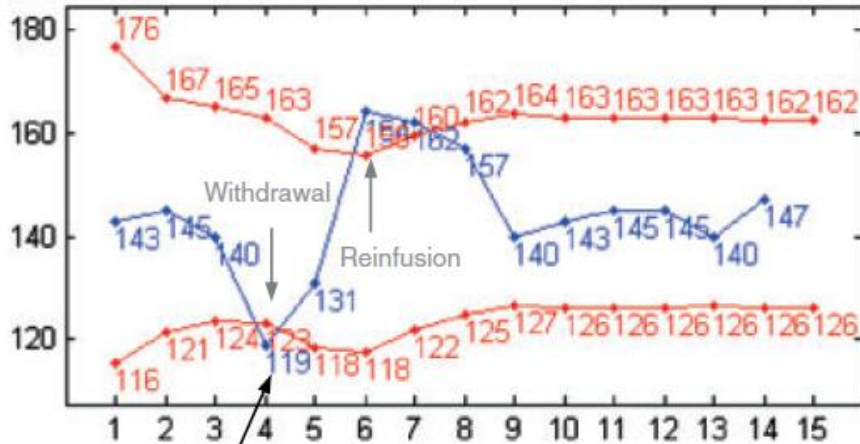


Biological passport: EPO/ ESA abuse

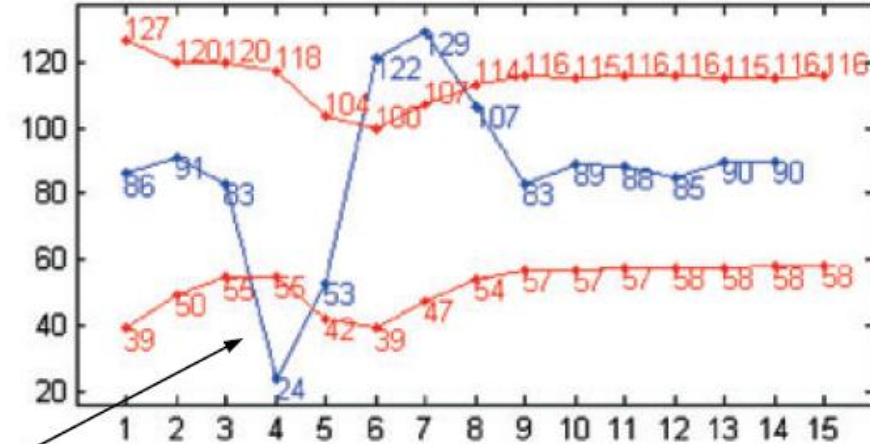
The samples 2-10 were taken on a regular base over a period of ~8 weeks.

Beyond the passport

Haemoglobin concentration (g/l)



OFF Score

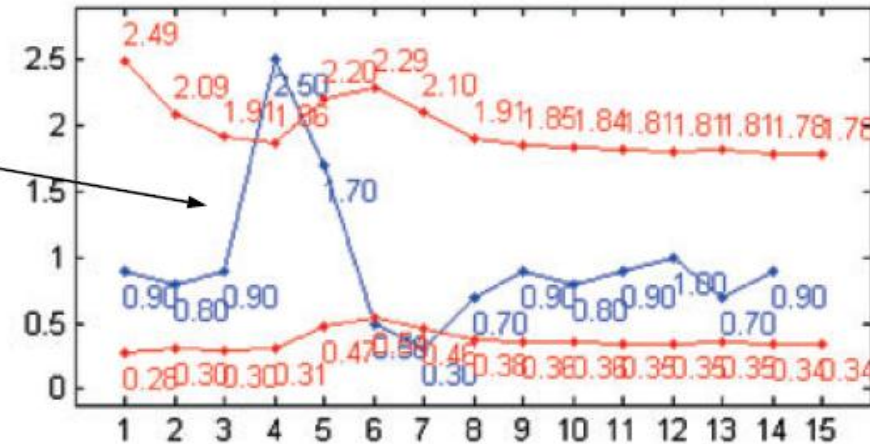


Large variation in Haemoglobin concentration after blood withdrawal and reinfusion.

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.

[Drug Test Anal.](#) 2012 Nov;4(11):846-53.
[Schumacher YO](#)¹, [Saugy M](#), [Pottgiesser T](#), [Robinson N](#).

Beyond the passport

- Competition schedule
- Training schedule
- Altitude training
- Whereabouts
- Curve of performances
- Lab APMU Expert's investigations
- Targeting system



Improve the global efficiency of the passport

Sample collection number

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The Prevalence of doping / Clean sport

IAAF Blood tests: 2001 - 2009

Tool to measure
the prevalence of blood doping

[Clin Chem.](#) 2011 May;57(5):762-9.

Prevalence of blood doping in samples collected from elite track and field athletes.

[Sottas PE](#)¹, [Robinson N](#), [Fischetto G](#), [Dollé G](#), [Alonso JM](#), [Saugy M](#).

	n ^o	Occurrence
Athletes	7289	Number: 2737
Sex	7289	Male: 55%
		Female: 45%
Competition	7287	Pre: 71%
		In: 6%
		Out: 23%
Age	6266	<19 years: 6%
		19–24 years: 28%
		>24 years: 66%
Nationality	6597	A: 9.8%
		B: 7.3%
		C: 6.1%
		D: 4.7%
		E: 4.0%
		F: 3.9%
		G: 3.8%
		Other: 140 <3.7%
Ethnicity	3487	White: 63%
		Asian: 9%
		African: 27%
		Oceanian: 1%
Sport	6328	Endurance: 79%
		Nonendurance: 21%

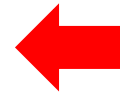
The Prevalence of doping / Clean sport

Table 2. Period prevalence estimates of abnormal blood profiles in elite track and field athletes.

	n ^a	Prevalence M1, % ^b	Prevalence M2, %
Males	4028	12 (10–15)	12 (10–15)
Country A	205	48 (35–63)	78 (54–99)
Country B	352	3 (1–11)	1 (0–2)
Country C	257	23 (15–30)	28 (17–36)
Country D	208	6 (3–19)	5 (0–17)
Country E	160	18 (11–30)	18 (7–28)
Country F	148	6 (1–25)	2 (0–22)
Country H	160	39 (20–54)	51 (21–87)
Females	3261	18 (15–21)	18 (15–21)
Country A	445	46 (35–58)	50 (35–68)
Country B	130	8 (4–34)	2 (0–11)
Country C	147	12 (4–20)	14 (1–28)
Country D	103	1 (0–11)	0 (0–3)
Country E	106	11 (7–20)	8 (1–14)
Country F	110	6 (3–19)	0 (0–13)
Country H	65	36 (13–62)	36 (5–66)

Blood tests 2001-2009

Mean: 12%
Country A: 78%
Country B: 1%



Prevalence M2:
 Micro-doses of EPO

[Clin Chem.](#) 2011 May;57(5):762-9.

Prevalence of blood doping in samples collected from elite track and field athletes.

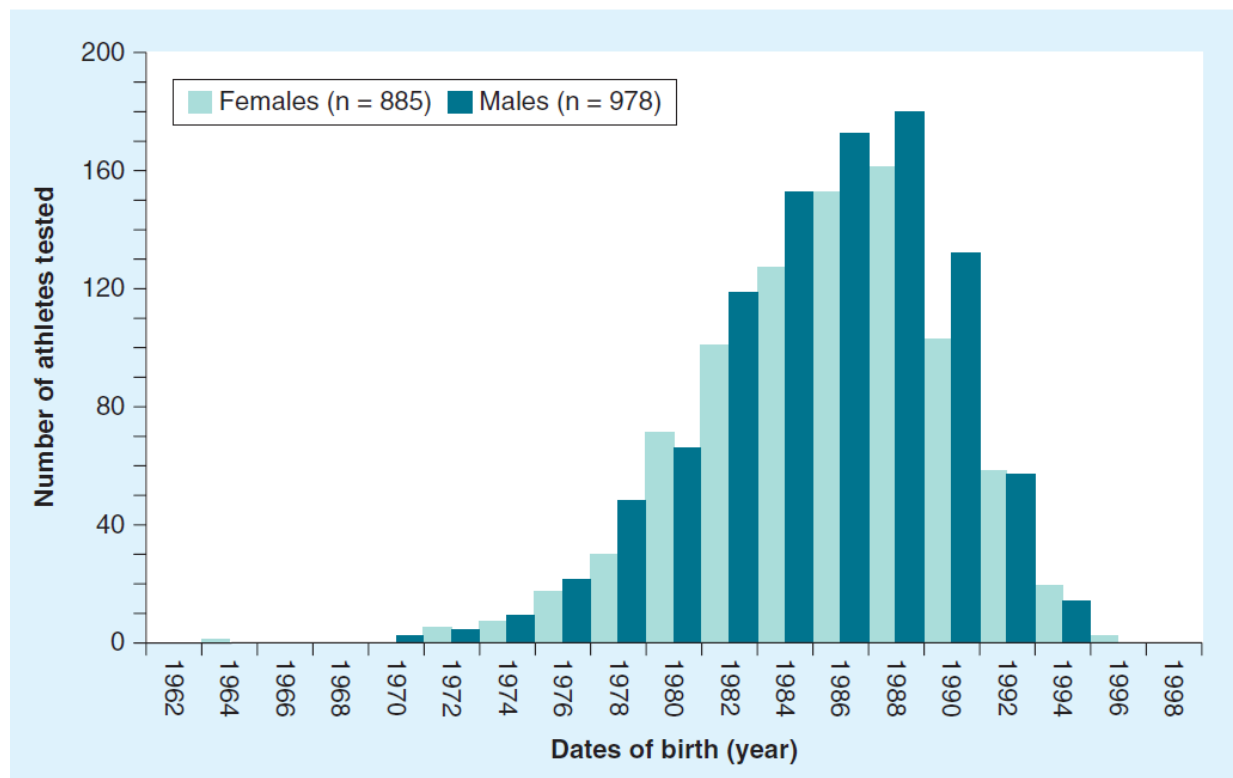
[Sottas PE](#)¹, [Robinson N](#), [Fischetto G](#), [Dollé G](#), [Alonso JM](#), [Saugy M](#).

The Prevalence of doping / Clean sport

2011 IAAF World Championships in Daegu: blood tests for all athletes in the framework of the Athlete Biological Passport

Neil Robinson¹, Gabriel Dollé², Pierre-Yves Garnier² & Martial Saugy^{*1}

[Bioanalysis](#) 2012, Vol. 4, No. 13, Pages 1633-1643



**WADA-IAAF-
LAD
Collaboration**

Figure 3. Gender-dependant distribution of the year of birth of the athletes.

The Prevalence of doping / Clean sport

Endocrine Research

Serum Androgen Levels in Elite Female Athletes

Stéphane Bermon, Pierre Yves Garnier, Angelica Lindén Hirschberg, Neil Robinson, Sylvain Giraud, Raul Nicoli, Norbert Baume, Martial Saugy, Patrick Fénichel, Stephen J. Bruce, Hugues Henry, Gabriel Dollé, and Martin Ritzen

Table 1. Age and Androgenic Parameters in the Studied Population

	n	Median	P25-P75	Minimum	Maximum
Age, y	849	26.0	23.0–29.0	16.0	47.0
T, nmol/L	849	0.69	0.50–0.93	0.01	29.30
DHEAS, μ mol/L	849	4.21	2.82–5.86	0.40	15.40
A4, nmol/L	849	3.32	2.51–4.40	0.47	18.85
SHBG, nmol/L	849	61.00	43.40–83.70	5.66	573.00
FT, pmol/L	849	8.20	5.34–12.18	0.12	469.28
LH, IU/L	849	3.53	1.84–6.24	0.10	88.40
FSH, IU/L	849	4.12	2.55–5.67	0.10	65.90
After removal of 10 athletes with confirmed DSD and/or doping					
Age, y	839	25.0	23.0–29.0	16.0	47.0
T, nmol/L	839	0.69	0.50–0.91	0.01	11.90
DHEAS, μ mol/L	839	4.23	2.80–5.86	0.40	15.40
A4, nmol/L	839	3.32	2.49–4.40	0.47	18.85
SHBG, nmol/L	839	61.40	43.70–84.20	5.66	573.00
FT, pmol/L	839	8.06	5.31–11.97	0.12	242.70
LH, IU/L	839	3.53	1.81–6.25	0.10	88.40
FSH, IU/L	839	4.10	2.51–5.64	0.10	65.90

Data are presented as median [25th percentile (P25) to 75th percentile (P75)], minimum, and maximum.

The Prevalence of doping / Clean sport

Evaluation of longitudinal steroid profiles from football players in UEFA competitions between 2008 and 2012



A Collaborative and retrospective study between
12 European WADA accredited Laboratories

The Prevalence of doping / Clean sport

■ EURO 2008

- Austria (OOC) and Switzerland (IC)
- Harmonization of the steroid profile quantification
 - Solid basis for data comparison



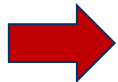
■ EURO 2012

- Poland (OOC and IC)
- Close collaboration with austrian and swiss antidoping labs
 - Possibility to compare results between both competitions



■ Other european competitions

- UEFA EURO Qualifiers, UEFA Champions Leagues, UEFA Europa League



Significant database to valorize !

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Pharmacokinetic / Re-analyses

Example of Cocaine case: a rider was found positive with 181 ng/ml BZE and 45 ng/ml MeE.



Rider explanation:

Mate de coca from his
Mother in law in Columbia

Urine test: 28 hours after intake

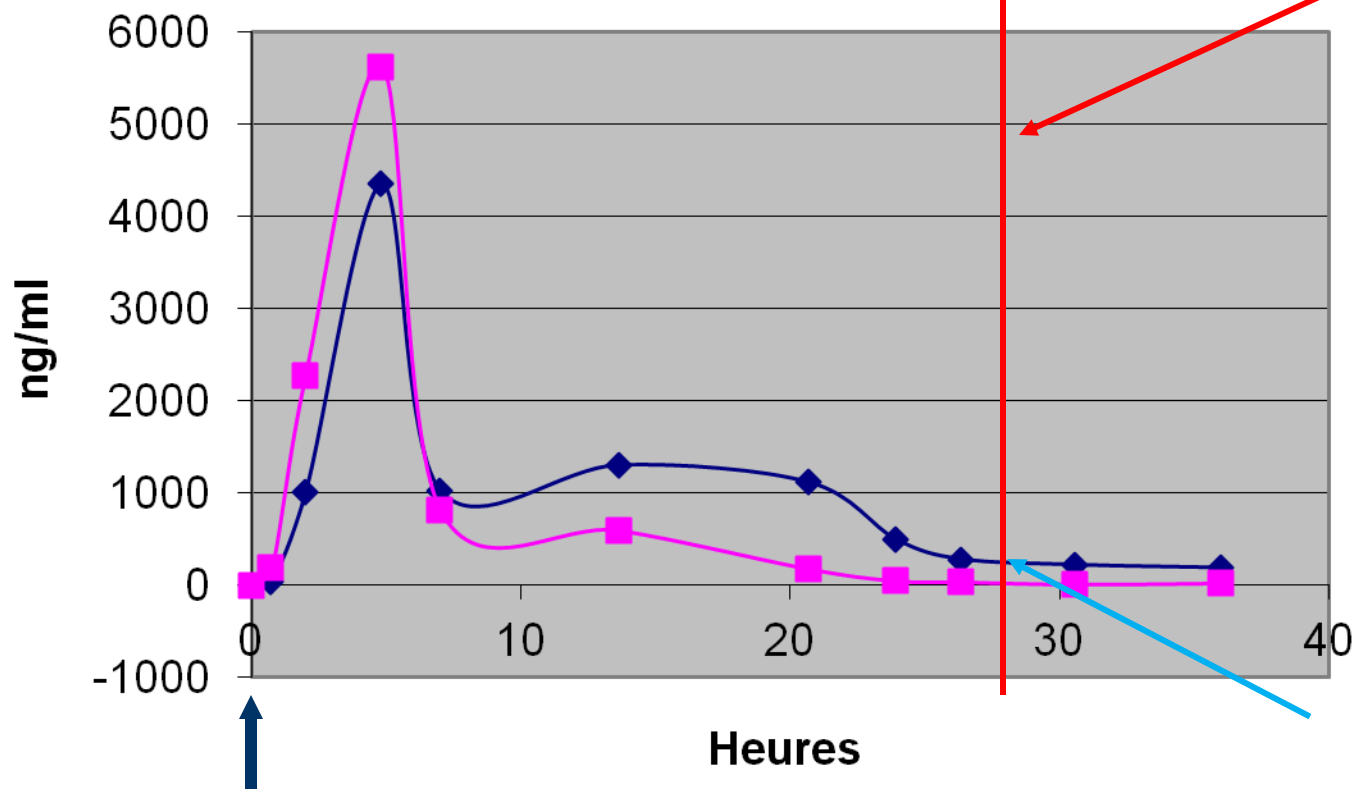
Plausible?
Pharmacokinetic data can help

Pharmacokinetic / Re-analyses

1 Tea bag in 200 ml d'eau

Excretion study with a volunteer

RIDER
181 Be
45 Me



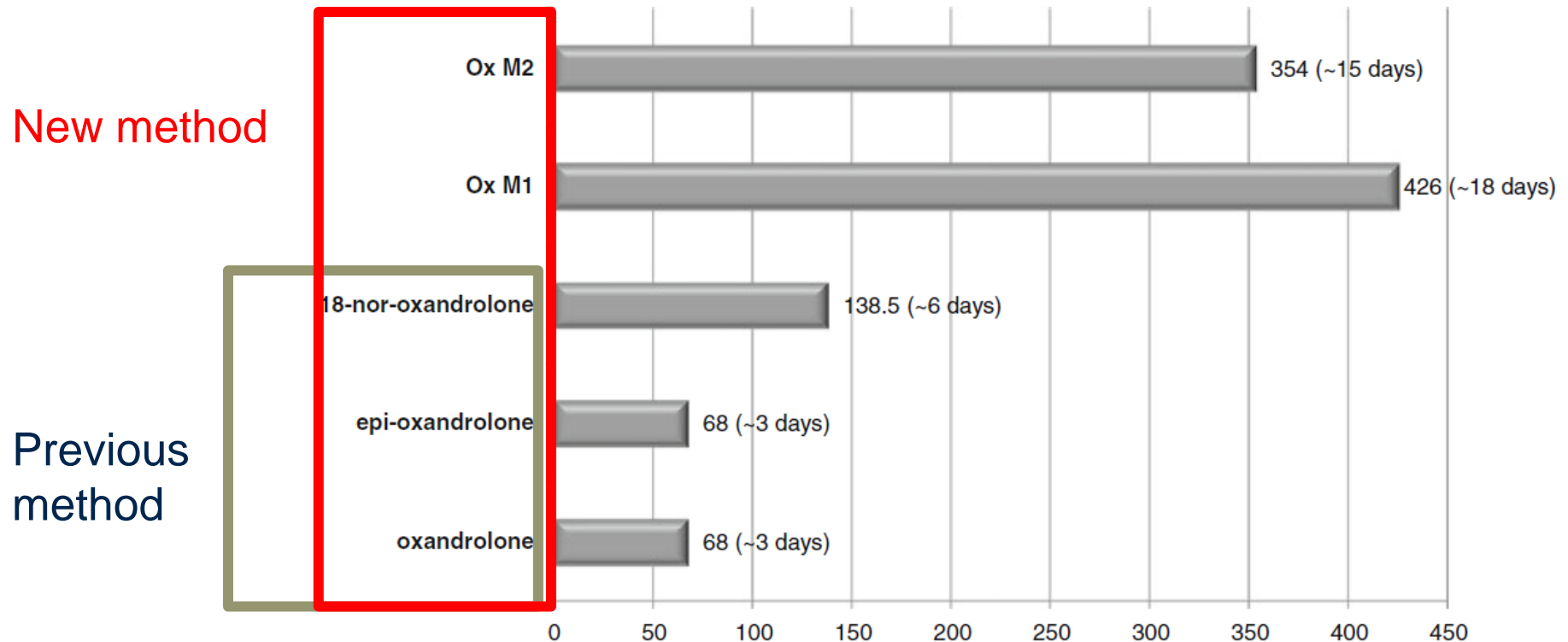
Volunteer
250 Be
30 Me

1.3 mg of cocaine

Pharmacokinetic / Re-analyses

Why should we re-analyze the stored samples:

Because the new methods are more sensitive and new metabolites are detected



Example of Oxandrolone

[Guddat et al, Anal Bioanal Chem. 2013 Oct;405\(25\):8285-94](#)

Pharmacokinetic / Re-analyses

Why should we re-analyze the stored samples:

Because the new methods are more sensitive
and new metabolites are detected

Yes, but: When ?

Do we know if there is a deterrent effect of the re-analyses
and is that any relation with the time after the test.

Yes, but: How?

We need a specific technical document
for the re-analyses to harmonize, to target, to decide

Yes, but: How much?

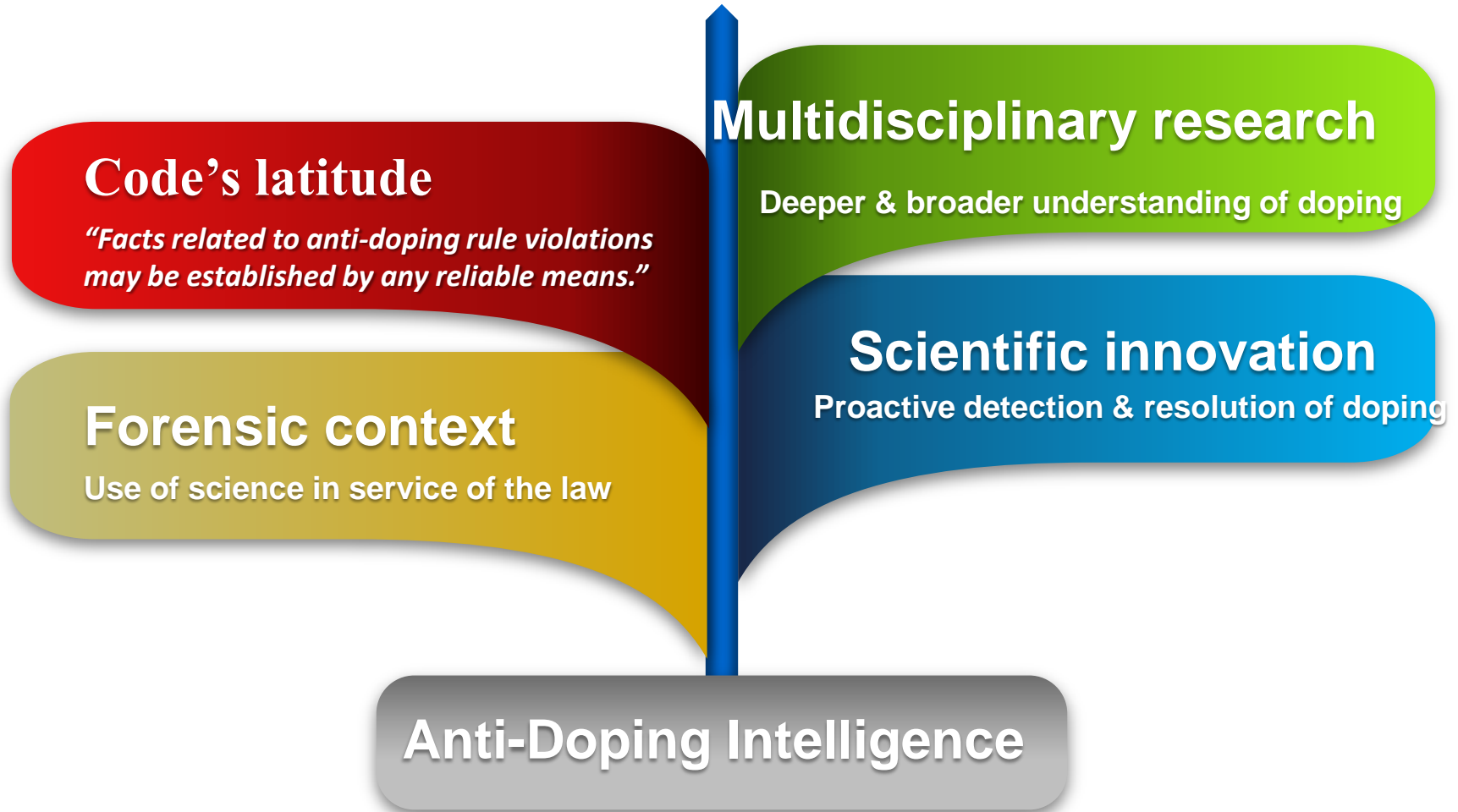
What is the real ratio cost-benefit of the process
long term storage/re-analyses

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Where do we go?

Ways for improving the effectiveness of Anti-Doping



Acknowledgements

To the Turkish Olympic Committee
and to WADA



And to the **LAD Team**