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Implementation of the biological passport: The experience of the International Cycling Union

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The concept of the biological passport is to evaluate, on an individual and longitudinal basis, the effects of doping substances and prohibited methods – blood doping and gene doping – on the body. Indirect biological markers can be measured and used to establish an individual's biological profile, when variations in an athlete's profile are found to be incompatible with physiological or medical conditions; a disciplinary procedure may be launched on the presumption that a prohibited substance or method has been used. As such, an athlete with a biological passport is his or her own reference.

The International Cycling Union (UCI) launched the biological passport programme in January 2008 in cooperation with the World Anti-Doping Agency (WADA). The UCI programme includes more than 850 athletes. These athletes are subject to urinary and blood anti-doping tests both in- and out-of-competition several times a year. Almost 20 000 samples were collected in 2008 and 2009.

In this article, the real-time process from sample collection to the opening of a disciplinary procedure is described. The establishment of this large-scale programme is discussed; the modalities which have to be applied and the difficulties encountered are presented. As for the results, some examples of normal and abnormal profiles are illustrated and indirect deterrent advantages of the programme are shown. Suggestions to improve the efficacy of the fight against doping through the implementation of the biological passport are discussed.

Keywords: biological passport; blood doping; indirect markers

Introduction

Blood doping is one of the most efficient forms of doping in endurance disciplines due to the induced increase in maximal oxygen uptake[1] (VO2max). Since the end of the 1980s, recombinant human erythropoietin (rhEPO) has been administered to athletes for the purpose of improving maximal aerobic power by augmenting the maximal oxygen-carrying capacity of blood.[2] Sporting authorities first addressed this issue by including rhEPO on the list of forbidden substances in 1990, then by promoting scientific studies aimed at detection of this potent illicit drug. Two directions in detecting rhEPO use at this time were being explored:[3–5] direct detection of rhEPO in urine (a method based on immuno-electrofocusing, validated and introduced in 2000)[6] and the indirect detection method,[4–7] where illicit use is marked through different haematological parameters known to be influenced by the administration of rhEPO and other blood doping agents.

To dissuade athletes from using the then undetectable rhEPO, some international federations (International Ski Federation [FIS], International Biathlon Union [IBU], International Skating Union, ISU) International Cycling Union [UCI] decided to introduce the so-called 'no-start rule'. This rule aimed at preventing athletes from competing when their hematocrit (HCT) or hemoglobin (HGB) values were higher than the established limit. For this purpose, a population limit was applied, the same limit being valid for all athletes.

In March 1997, the UCI health test programme was launched and athletes were subjected to tests at or during competitions but not out-of-competition. Throughout the years, the programme evolved with scientific and technologic improvements. New parameters were added (percentage reticulocytes (RET%), Off-hr score,[4] free plasma hemoglobin) and new limits for the no-start were introduced. In 2006, UCI decided to establish an external quality control programme for laboratories involved with 'in-the-field' hematological analysis. This programme, supervised by the Swiss Center for Quality Control (CSCQ) assessed on a monthly basis the reliability of the hematological analysis performed by the different laboratories. CSCQ concluded that in-the-field analysis provided similar results to those performed in a laboratory setting. Furthermore, differences in results were within the expected range. The results from the different machines proved to be comparable and thus an important stage was achieved in respect to the longitudinal evaluation of an athlete's results.

The Athlete's Biological Passport

Several scientists were simultaneously developing different strategies related to the indirect detection of blood doping. The Australian[5–8] and the Lausanne[5–8] groups have intensively explored the possibility of introducing a multiparametric marker approach that takes into account different factors known to influence the biological variability, such as the technology applied, the athlete's gender, age, ethnic origin, and sports discipline. Contrary to what was applied at that time by the sporting authorities, a new concept was also taken into consideration: individual limits instead of population limits. As such, one could use the athlete's previous measurements as basal levels with each athlete becoming his or her own reference. The application of subject-specific reference ranges corresponds to what some authors had suggested at the

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beginning of the last decade.[5] An initial practical application called the 'Swedish Blood Pass Project' was developed in 2005 by Berglund.[6]

All of these important scientific achievements convinced WADA to evaluate the feasibility of introducing an athlete's passport. The first step was the creation, in 2006, of a Haematological Working Group composed of scientists and representatives of international federations. These experts concluded that WADA could proceed with the implementation of an *Athlete's Haematological Passport*. They recommended measurement of indirect parameters (namely haemoglobin concentration and Off-hr score) in- and out-of-competition in order to generate profiles that would be computed with the Bayesian statistical model developed by the Lausanne Anti-Doping Laboratory. Each value, as well as the entire sequence of results, was to be evaluated, in the event that an athlete's profile was found to be incompatible with typical physiological or medical conditions, a disciplinary procedure against the athlete could be initiated based on the presumption that a prohibited substance or method had been used. It was also agreed that standardized protocols should be established and followed in order to decrease the variability due to pre-analytical and analytical conditions. These protocols would explain how blood samples should be collected, transported, and analyzed.

UCI's Pilot Project

A meeting took place in Paris in October 2007, between WADA, the French sporting authorities, and the cycling family. There it was decided that UCI would launch, in 2008, a pilot project for the implementation of the haematological module of the *Athlete's Biological Passport*.

Such a commitment required that several conditions be fulfilled prior to the initiation of the programme. First, it was necessary to define the target population to be tested, during in- and out-of-competition. In the UCI programme, athletes were enrolled by teams. All male road ProTour Teams (first division) and those ProContinental Teams (second division) applying for a wild card in order to have access to the major races, were automatically included. Additionally, some individual teams or athletes were also integrated. This brought the number of athletes to 804 in 2008 and to 848 in 2009.

Whereabouts information

It is essential to have easy, manageable whereabouts data (information regarding an athlete's present geographical location), so that tests can be planned anytime throughout the year. We therefore encouraged all athletes to provide this information through ADAMS, the web platform which has been developed by WADA. We also accepted other electronic formats of whereabouts (i.e. those coming from athletes whose national anti-doping organization had chosen to use alternative systems). At the beginning of the 2008 and the 2009 season, UCI staff visited all of the teams at their training camps, presented the ADAMS system, and proposed phone or e-mail assistance if needed. We facilitated the ADAMS implementation by publishing a guide and financing translations to other languages (Italian and Flemish, in association with the Dutch National Anti-Doping Organisation). Teams were also involved in the use of ADAMS by directly entering riders' competition schedules and training camps and through assistance in fulfilling this important requirement. These different actions enabled the successful management of whereabouts. Only a very low number of whereabouts failure or missed tests were recorded.

Sample collection

The main part of the programme is of course sample collection. Up until 2008, UCI would collect and analyze blood health samples on the site of the competition prior to the departure of the race so that the athletes were notified of the decision about their 'aptitude' or 'inaptitude' to compete. Presently, the *Athlete's Biological Passport* requires that athletes be tested not only at competition sites but also out-of-competition. It is therefore necessary to have a large pool of Blood and Doping Collection Officers (BCOs and DCOs) who can conduct tests at the athletes' homes, at training camps, or at competitions. UCI has signed agreements with organizations specialized in the collection of doping samples (International Doping Tests and Management (IDTM) and Physical Work Control (PWC)) that are mainly used for out-of-competition tests, whilst UCI's DCOs/BCOs are in charge of the majority of the pre- or in-competition tests.

In January 2010, WADA published the *Athlete Biological Passport Operating Guidelines*[7] and technical documents that define the modalities of samples collection:

- Samples can only be collected 2 h after a physical effort (training or competition).
- The athlete has to remain seated for at least 10 min prior to providing a sample.
- The athlete should be questioned about specific issues, like altitude (natural or simulated), blood losses, donations, and transfusions.

UCI has progressively taken these different elements into account because the documents were only validated in December 2009.

For the purpose of measuring the hematological parameters of the biological passport, one A tube (3 ml tube containing EDTA as anti-coagulant) is sufficient as it is not required to conduct additional analysis. Nevertheless, in order to increase the deterrent effect and the efficiency of the programme, a collection of two EDTA tubes (tube A and tube B) should be obtained as, in case of an abnormal result, this enables the potential for the immediate request of an anti-doping test (in whole blood or plasma) for the detection of homologous blood transfusion (HBT), Continuous Erythropoiesis Stimulating Agent (CERA), or Hemoglobin Based Oxygen Carriers (HBOC).

Finally, at least three blood tests are necessary in order to evaluate the entire sequence in addition to the single value. At the beginning of the programme in 2008 we planned to collect a total of 10 blood and 4 urine samples for each athlete both in- and out-of-competition. In 2009, this scheme would be the same for the new riders entering the programme, while the older ones would undergo a reduced number of tests (6 blood and 3 urine tests), unless other reasons (i.e. abnormal profiles, sport performance) dictated otherwise.

Samples analysis

Once collected, samples should be stored and transported to the closest laboratory in refrigerated conditions (2°–12°C). In fact, samples have to be analyzed within 36 h of their collection. The drawback of this procedure is that it increases the costs incurred through transportation.

Table 1. List of WADA-accredited anti-doping laboratories which can perform analysis for the haematological module of the *Athletes' Biological Passport*

Barcelona (Spain)	Madrid (Spain)
Beijing (China)	Montreal (Canada)
Bloemfontein (South Africa)	Moscow (Russia)
Cologne (Germany)	Oslo (Norway)
Delhi (india)	Paris (France)
Dresden (Germany)	Rome (Italy)
Ghent (Belgium)	Salt Lake City (USA)
Lausanne (Switzerland)	Seibersdorf (Austria)
Lisbon (Portugal)	Sydney (Australia)
London (Great Britain)	Tokyo (Japan)

When the programme was launched, only a few accredited laboratories were available: those that UCI had been using since 2003. Laboratories have to respect some preliminary conditions in order to be accredited to perform hematological analysis:

- They all have to use the same technology, in order to decrease the variability associated to reticulocytes measurement.
- They must be part of a common external quality assessment.
- The same procedures must be applied when calibrating the machines (quality control checks, fresh blood samples analysis) and analyzing the samples.

From 2008, WADA started to accredit additional anti-doping laboratories for the analysis of the parameters hematological module of the biological passport. Today, 20 WADA-accredited laboratories (Table 1) can perform this specific analysis. This has a direct impact on the programme by reducing the costs of transport and improving efficiency by allowing simultaneous anti-doping tests on the blood sample or on urine samples collected at the same time.

The parameters that are measured, within the hematological module, are:

- Red blood cells (RBC)
- Hematocrit (HCT)
- Haemoglobin (HGB)
- Mean Corpuscular Hemoglobin (MCH)
- Mean Corpuscular Hemoglobin Content (MCHC)
- Mean Corpuscular Volume (MCV)
- Off-hr score (HGH - 60$\sqrt{}$RET%)
- Absolute number of reticulocytes (RET#)
- Reticulocytes percentage (RET%)

Only the HGB and Off-hr score are taken into account by the Bayesian model in order to define a possible anti-doping rule violation.[7]

Samples collected

During the first year, UCI faced a major challenge: to find resources, both human and financial, in order to conduct the programme outlined above. At the end of 2008, fewer samples than expected were collected because of a lack of financial means (not all the money promised by some stakeholders was received) and also because the different groups of blood collectors could not respond to the huge demand imposed by the programme. It should not be forgotten that before 2008 only a few ADOs were collecting a limited number of out-of-competition blood samples.

Table 2. Anti-doping tests conducted within the UCI Biological Passport Programme 2008/2009

		2008	2009
In-competition tests	Urine	1463	1672
	Blood	492	601
Out-of-competition tests	Urine	1452	2165
	Blood	4997	6165
Total anti-doping tests		8404	10 603

The situation has progressively improved. More BCOs have been made available, so that in 2009, the number of samples collected by UCI increased by almost 26% to 10 603 (Table 2), i.e. 12.6 samples per athlete. The majority of the samples were collected out-of-competition (which includes real out-of-competition but also pre-competition tests).

It is important to highlight two aspects: all of the 2165 out-of-competition urine samples were tested for rhEPO, and 851 blood passport samples were tested for CERA using the immunological assay screening test.[8]

Real-time process

As previously mentioned, ADAMS is the web platform which allows all programme data to be centralized and shared by all of the stakeholders. In addition to whereabouts (and therapeutic uses exemptions or declaration of use), testing missions are planned using ADAMS. The testing authority, by creating the mission order, defines which athlete should be tested; where and when the test should take place; who the sample collection authority should be; what kind of sample should be collected; to which anti-doping laboratory the sample should be sent; and what kind of analysis should be requested. Once the sample has been collected, the doping control form is entered by DCOs and BCOs and the laboratory results are also transferred from the analyzer. The latter are available within a short period allowing the testing authority to react rapidly when an abnormal result is received. They can immediately ask to conduct an anti-doping test on the collected sample (for CERA, HBT, or synthetic hemoglobin) or decide to collect an additional target test. The only part that is not automated is managed by the Athlete Passport Management Unit (APMU) which is in Lausanne and is financed by the Laboratoire d'Analyse du Dopage (LAD) and WADA. APMU is responsible for the anonymous extraction of data from ADAMS and for computing this information into the ABP software that generates an individual encoded athlete's ABP profile. This anonymous profile is what the experts receive for their evaluation and recommendation.

The experts were chosen by UCI and WADA. All are qualified in the field of hematology (either clinical or laboratory), sports medicine, exercise physiology, or blood doping. It is important to include experts with different knowledge, so that a profile can be evaluated from different perspectives.

Each week, 10 to 15 updated profiles are sent to the experts for review. In these profiles the Bayesian adaptive model has identified the Hb or Off-hr score abnormal with a 99% probability (either for the single measurement as a function of previous results or for the complete sequence) or with normal or lower levels of probability. The aim is that all profiles are submitted to the experts' evaluation.

At any time, and in order to better evaluate the anonymous ABP profile, the experts may request additional information, such as the

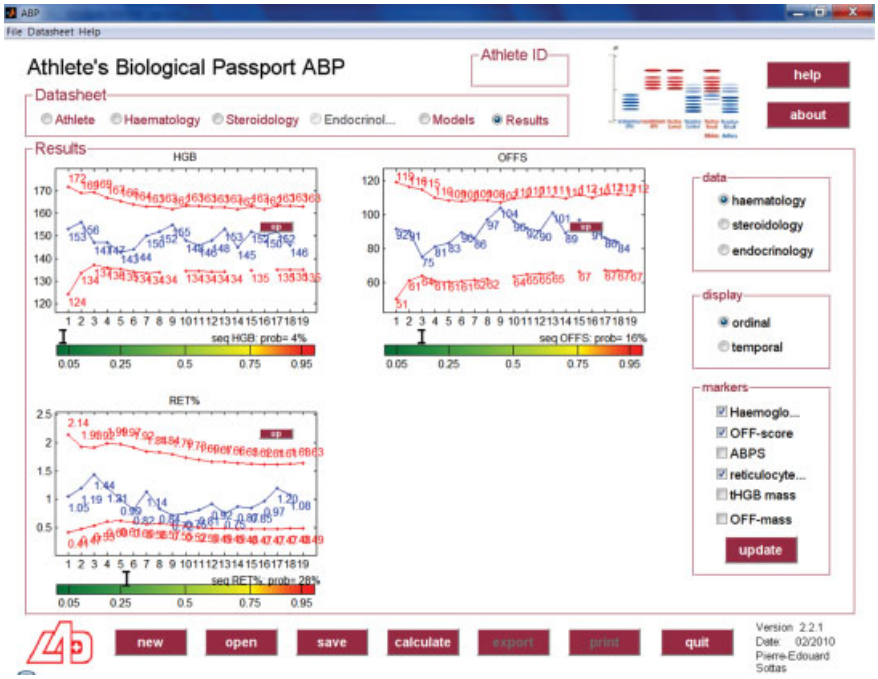

Figure 1. ABP profile of an athlete considered as normal.

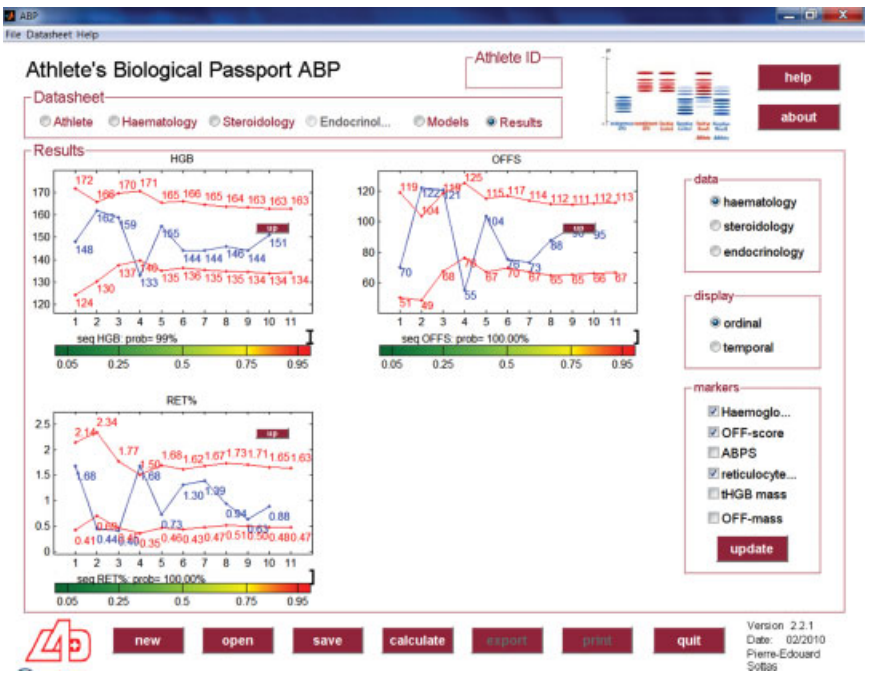

Figure 2. ABP profile of an athlete considered as suspicious.

athlete's whereabouts, competition schedule, strange journeys, laboratory documentation packages, or medical information. Once evaluated, the experts may formulate a number of recommendations. The profile can be judged as normal (Figure 1) or as suspicious.

If it is suspicious, targeted tests can be conducted to detect forbidden substances and methods or to strengthen the suspiciousness of the profile. If the experts agree that the profile is abnormal and consistent with a pattern of blood doping (Figure 2) recommendations are made to UCI to undertake the procedure for suspected anti-doping rules violation. In fact, the 2009 World Anti-Doping Code enables the possibility of sanctioning an athlete for the use of doping based on an established abnormal blood or urine profile.

The procedure foreseen by the WADA guidelines requires that the athlete be:

- informed that the anti-doping organization (ADO) is considering bringing an anti-doping rule violation against him or her;
- given a copy of any document made available to the expert;

- allowed to rebut the allegation by letting him or her provide his or her own explanation for the abnormal profile. The athlete can, for instance, demonstrate that the results were the consequence of a pathological condition.

This explanation is further reviewed by the experts who must finally and unanimously decide if, in their opinion, there is no known reasonable explanation for the abnormal blood profile other than the use of a prohibited substance or method. In such a case, the three experts sign a statement asserting that the profile provides 'convincing evidence of the use of a prohibited method', and make an official recommendation to the ADO to open a disciplinary procedure against the athlete.

Based on UCI regulations, when a disciplinary procedure for an anti-doping rules violation procedure is opened, the athlete, his or her Team, the National Federation, the National Anti-Doping Organization (NADO) and WADA are informed and the National Federation is to take charge of the disciplinary proceeding. At this stage, no provisional suspension is enforced, although usually the Team decides to suspend the athlete from competition.

Results

In terms of results, the UCI Blood Passport programme for 2008 and 2009 can be evaluated on different levels.

Presently, more than 800 top-level road athletes are providing whereabouts in an electronic format, mainly ADAMS, so that all ADOs with authority can conduct out-of-competition tests all year long. This is probably the largest registered testing pool for one single discipline among all international federations and plays an important role in terms of deterrence, as will be discussed later.

In terms of success, we have opened 9 disciplinary procedures based on the haematological profiles obtained in 2008 and 2009. Four of these cases have already been adjudicated by the first degree instance and no appeals have been launched so far. It is also worth mentioning that, for some of these athletes, additional evidence was available: in one case, we reviewed all of the EPO tests conducted in previous years and requested the lab re-analyze a urine sample, which was finally considered positive for Dynepo; in another case, a single biological passport sample revealed the presence of CERA.

On the other hand, athletes with a suspicious haematological profile are subjected to targeted out-of-competition tests. Twenty-two athletes were found positive as a result of these targeted tests: 20 for an Erythropoiesis Stimulating Agents (ESA) (9 in 2008, 11 in 2009) and 2 for exogenous anabolic steroids.

The efficacy of the programme can also be assessed through other means; for instance, by observing the values of RET% measured from 2001 to 2009 (Figure 3). The percentage of reticulocytes is one of the most reliable markers of blood doping.[9] After an ESA administration (boosting period – ON phase), RET% showed a marked increase (blue values in Figure 3) up more than 2%. Contrary to this, during the maintenance and wash-out period (OFF-phase), they decrease to even lower values than the level measured prior to the ESA or transfusion therapy (green values in Figure 3). Figure 3 indicates that from 2001 to 2007 an almost constant percentage of samples (around 10%) had RET% results in the extreme ranges (<0.4% or >2.0%). Since the introduction of the biological passport this number has dramatically decreased to 2–3% notwithstanding the fact that samples were also collected out-of-competition when doping products are more likely to be taken. Additionally, very extreme values (<0.2% or >2.4%) have

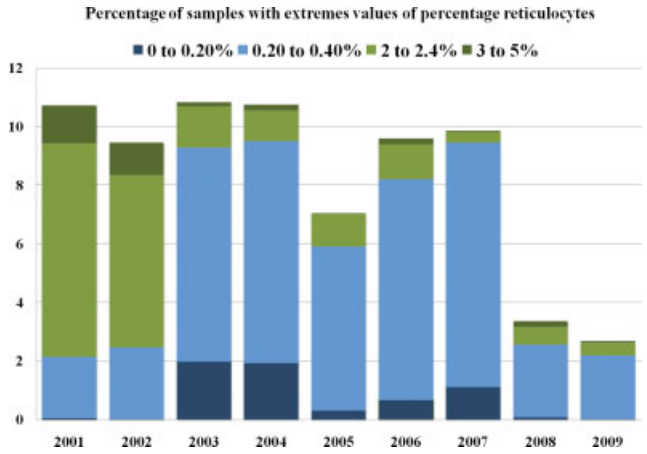

Figure 3. Percentage of sample whose RET% values are extremes: low values (blue) are typical of an OFF phase (previous intake of ESA or blood transfusion), while high values (green) indicates an ON phase (recent use of ESA or haematological disease).

Table 3. Biological passport lab results. Profile of an athlete found positive for ESA

Test	HCT	HGB	MCH	MCHC	MCV	Off	RBC	RET#	RET%
1	59.6	19.6	29.6	32.9	89.9	106.20	6.63	0.1485	2.24

completely disappeared since 2009. This data suggests that the behaviour of the peloton has changed in relation to the use of blood doping agents which confirms the feedback we get when discussing the topic with the 'field'.

Another important deterrent effect of the biological passport is that more and more athletes are required to provide their results when negotiating a contract with a new team or when they are selected to compete in the National Team. We are aware of situations where new teams or the National Federation have refused to hire or select athletes because of their suspicious profile.

Examples

We now present two examples where the passport has led to targeted anti-doping tests which ended in a positive result for ESA.

In the first case (Table 3), the athlete tested for the first time showed extremely high values of HGB and RET%. Because we do not have previous results to make a comparison, we can assume two possible origins: either the intake of ESA (a blood transfusion is less likely) or the athlete is suffering from a form of erythrocytosis of unknown etiology. The latter hypothesis can easily be evaluated by conducting additional target tests to see if the high blood values are constant. If so, they are an indication of a possible medical condition that needs to be investigated. In the meantime, a CERA test should be performed on the collected plasma together with an EPO test if urine is available. Nevertheless, such an abnormal result will have a high influence on the future haematological profile (because of the variations) and it is realistic to imagine that such an abnormality will provide enough evidence to open a disciplinary procedure for an anti-doping violation case. This was not necessary because the urine sample collected at the same time as the blood test was reported positive for ESA.

Table 4. Biological passport lab results. Profile of an athlete found positive for ESA

Test	HCT	HGB	MCH	MCHC	MCV	Off	RBC	RET#	RET%
1	44.9	15.2	29.6	33.9	87.5	62.20	5.13	0.1149	2.24
2	48.8	15.8	29.8	32.4	92.1	98	5.3	0.0530	1.0
3	48.8	16.2	30	33.1	90.6	98.79	5.4	0.0598	1.11
4	51.8	16.8	29.5	32.4	90.9	107.7	5.70	0.0576	1.01

In the second case (Table 4), the athlete's first test showed a high value of RET%. It was decided to submit him to targeted urine and blood tests. The HGB and RET% variations in the following tests were consistent with a maintenance treatment of ESA or the use of blood transfusion. Finally, the athlete was found to be positive for ESA.

Conclusions

The introduction of the *Athlete's Biological Passport* is clearly a major step forward in the fight against doping. It allows the efficient combination of two different but complementary strategies. The old traditional method for detecting a forbidden substance in a bodily sample can now be coupled with a more subtle detection of the biological consequences induced by these drugs. The downside is the increase of costs due to the particularity of these tests where blood samples have to be handled differently from normal urine samples. Furthermore, the management of results is more complex than in traditional anti-doping tests.

UCI's experience has shown that, in order to increase efficiency at equal costs, the option of only collecting a whole blood sample should be discarded in favour of the classical A and B samples. The athlete will not know if the sample is used for profiling or for detection. It is a permit to directly conduct an anti-doping test to detect a forbidden substance or method when the profile is suspicious. Furthermore, the simultaneous collection of other biological samples (serum, urine) enables additional tests (EPO, hGH, urinary steroid profile). In this perspective, the use of anti-doping laboratories would be preferred, taking into account the logistical difficulty of having to analyze a sample within 36 h of collection.

In the future, blood samples could be used to analyze new biological markers (IGF1, P-III-P) or to carry out completely new tests in the field of proteomics, metabolomics and others.[10]

Following work pioneered by UCI and WADA, other ADOs are progressively introducing a biological passport programme: Anti-Doping Norway, International Association of Athletics Federations [IAAF], UK Anti-Doping, and others.

It seems that the *Athlete's Biological Passport* is just the first chapter of a new book that is going to be written in the coming years.

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