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Review article

Strategies to combat doping in cycling

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Abstract

The sport of cycling has been affiliated with doping for decades. Currently, the battle against doping has been intensified by the development and implementation of large-scale anti-doping programmes. The primary aim of this paper is, from a practical point of view, to describe the most efficient way of combating doping in cycling today.

Of major importance is sample collection from the cyclists, which must be intelligently scheduled according to the substances tested for. A prerequisite for this is an efficient "whereabouts" system, and the collaboration between different testing bodies. Samples must be collected and analysed according to strict prescriptions thereby reducing the analytical variations, which are an essential part of individual longitudinal data comparison. Blood screenings can be performed on-site in a mobile laboratory allowing rapid follow-up testing, while urine must be analysed in one of the World Anti-Doping Agency (WADA) laboratories. The reporting of the sample collection must be extended to include information on potential confounding pre-analytical factors and the reporting of the analysis and test results must be streamlined and extended to include more quantitative data i.e. from the recombinant Human Erythropoietin (rHuEPO) test. In addition, a high level of education of the riders and transparency of test results will demystify the anti-doping activities for the riders as well as the public. **Keywords:** doping, testing, cycling, analysis, athletes

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Introduction

The history of doping in cycling probably dates back to 1886, where an English cyclist is reputed to have died after drinking a cocktail of cocaine, caffeine and strychnine during a race from Bordeaux to Paris. Since then a large number of top-level riders have tested positive or admitted to doping during their careers, and the sport of cycling has inevitably been connected to doping during the last decades. A wide range of different drugs have been used by cyclists, from stimulants, such as amphetamine and cocaine in the early days, to anabolic steroids, recombinant Human Erythropoietin [rHuEPO], blood transfusions and human growth hormone (hGH).

The fight against doping began after the death of a Danish cyclist during the Olympic Games in Rome in 1960. The international cycling federation, the Union Cycliste Internationale (UCI) developed a set of rules and in 1967 a medical committee under the International Olympic Committee (IOC) was established with the aim of designing strategies to combat doping in sports¹. Small-scale tests for stimulants were introduced in 1964 and four years later, at the Olympic Games in Mexico City, a larger doping testing programme was developed. In 1997, the UCI launched a programme which included unannounced blood testing before competition, which was followed by the 'Monitoring Programme' in January 1999. The aims of these programmes were to 'protect the athlete's health, promote fair competition and improve the fight against doping'. A haematocrit (Hct) limit of 50% for males and 47% for females was introduced. If these limits were exceeded before the competition the riders were not allowed to compete.

In the aftermath of the Spanish doping scandal 'Operation Puerto' in 2006, different cycling teams initiated and funded their own anti-doping programmes, where external

experts were consulted, and in 2008 the UCI, as the International Skiing Federation had done 18 months previously, introduced a "biological passport". This passport is an individual document for each rider, in which the results of all anti-doping analyses (haematological parameters, urinary steroid profiles) are recorded. These data allow the individual haematological and urinary profile of each rider to be constructed and thereby, over time, create individual upper and lower limits instead of population-based limits. By publishing the numbers of collection on the UCI homepage, the public is invited to follow the testing progress. Specific results showing the variation in the numbers of tests per rider and the timing of these tests have not yet been published, but it is anticipated to do this in the very near future. These new initiatives have markedly increased the number of tests per rider per year, and professional riders are now faced with the most extensive doping testing programmes in the world of sport. As previously mentioned, the primary aim of this paper is, from a practical point of view, to describe the most efficient way of combating doping in cycling.

Sample scheduling

The time of sample collection and the analytical procedures used are two important issues that from a scientific as well as an economical perspective must be taken into consideration when developing an anti-doping programme.

The time of sample collection can roughly be split into the following four categories:

1. 'Pre-competition': Samples collected 48-12 hours before a race
2. 'In-competition': Samples collected 0-12 hours before a race, during a stage race, or 0-4 hours after a race.
3. 'Out-of-competition': Samples not belonging to Category 1 or 2.
4. 'Training camps': Samples collected during training camps



As for all sports, most doping testing in cycling has previously been performed 'in-competition', making it possible to collect a large number of samples within a short period of time. The problem with this kind of testing is that riders are well aware of being tested and therefore evade detection by ceasing their doping administration days before the event. For example, rHuEPO has a very short half-life and is therefore detectable in urine only for a short period of time after administration. On the other hand, the performance-enhancing effect is far more long-lasting than the detection period, and therefore the riders that use this drug will still benefit from the effects during competition²⁻⁴. Documents from 'Operation Puerto' support this testing method by revealing detailed doping calendars describing the exact dates, where riders were to administer doping agents to increase their performance during competitions but avoid being tested positive by the doping authorities. During competition, only undetectable drugs were administered while all other doping agents were taken 'out of competition' on specified days. Therefore to increase the sensitivity of doping testing, samples must, in general, primarily be collected unannounced and 'out of competition'. This is especially important when testing for the major doping groups such as anabolic steroids, rHuEPO and hGH. However, testing for relatively short-acting substances, like amphetamines, oral anabolics and haemoglobin-based oxygen carriers (HBOC), or long-detectable substances, like homologous blood transfusions, should be performed in-competition.

A prerequisite for an efficient unannounced out-of-competition testing programme is a well-designed database, where information on sample collection, test results and whereabouts of cyclists are shared between testing bodies. WADA has developed and implemented the Anti-Doping Administration and Management System (ADAMS) which manages these issues. Since the riders are subject to doping control from up to four different parties: National Anti Doping Organisations (NADO), International Cycling Federation (UCI), WADA and (in some cases) Internal Team Testing Programmes, all parties should be allowed access to this database, so that sample collection can be coordinated between all testing bodies and the "surprise" effect and efficiency of testing remains optimal. This reduces duplicate

testing and thus the costs of out-of-competition testing, which can be significant, when only one or a few samples are collected at distant places. Having a broad network of doping control officers (DCOs) scattered all over the world allows fast sample collection and reduction of travel costs. In this regard an efficient 'whereabouts system' is an essential part of sample collection. Anecdotal evidence suggests that riders travel to distant places to administer drugs in preparation for competitions and 'forget' to change their whereabouts during this period. Therefore WADA has decided that within a period of 18 months any three combinations of failure to file the required whereabouts information or missed tests, in which case athletes are not where they are supposed to be, constitutes an anti-doping rule violation. An alternative and more radical 'whereabouts system' would be to provide each rider with a Global Positioning System (GPS). This would ease the work of the DCOs, reduce the time and effort of reporting, and eliminate so-called 'no-shows', where riders are not where they reported they will be.

A less expensive way of collecting samples compared to out-of-competition testing is to do this during training camps. In contrast to many other team sports, where training is performed as a team, in cycling it is rather uncommon to have all riders gathered at the same place at the same time. Usually riders live and train by themselves or in smaller groups, and only come together a couple of times each year. During training camps it is possible to collect a large number of samples during a short period of time, which markedly reduces the cost per sample. With this in mind, one could argue that riders are well aware of being tested during training camps, and therefore will abstain from doping during this period. Nevertheless, testing during 'clean' periods is a keystone in longitudinal testing and the development of individual blood, steroid and EPO urine profiles, because it is probable that large individual variations are more likely to show up, if 'clean' samples are mixed with 'doped'.

Another vital aspect is the choice of which riders to test. Because of economical and logistical reasons it is not possible to test all riders under the UCI. Therefore a registered testing pool (RTP) must be developed. The UCI has included all riders on ProTeams and riders on Professional Continental Teams



who have entered as wild cards in their RTPs, which also contain top ranked riders other than road cyclists and riders who are 'of concern'. This seems a reasonable approach since the likelihood of top riders doping must be considered higher than lower level riders doping. Testing should not be done randomly but riders should be selected intelligently. The targeting should be based on individual race calendars, which give valuable information on when riders are scheduled to physically peak during the season. Special attention should be given to riders showing rapid performance improvement, or riding with teams, or persons with a history of doping.

Sample collection and analysis

During the collection procedure itself, DCOs must adhere to the International Standard for Testing⁵. From the moment that the rider is approached and during the collection procedure itself, the cyclist should be supervised at all times. This is of major importance when urine is collected because the non-invasive procedure allows for sample tampering; e.g. addition of very small quantities of protease has been shown to remove all traces of EPOs in urine⁶. In contrast to urine sampling, blood collection makes it impossible to tamper with samples during collection and the humiliating situation of the cyclist urinating in front of a stranger is also avoided. Therefore future doping tests should be blood-based if possible, as exemplified by the addition of a step of immuno-affinity to the method currently used for the detection of rHuEPO in urine, which has made it possible to detect rhEPO in serum².

After the samples have been collected, they must be handled and stored under strict conditions according to the type of analysis and human material collected, i.e. urine or blood. Urine samples must be kept chilled or frozen and transported to a WADA-accredited laboratory for analysis, thereby allowing a long storage period. On the contrary, blood is relatively unstable and whole blood cannot be frozen. Previously, blood tests have been considered as pre-emptive blood screens, which would lead to suspension from competitions for health reasons or be used for urine test targeting. However, during the last few years, blood tests have gained acceptance and with the introduction of the 'biological passport', the

UCI decided to sanction athletes for longer periods if non-physiological changes in certain blood parameters appear⁷⁻⁸. The rationale for this approach is that each rider has their own genetically determined levels around which they are allowed to fluctuate to a certain extent. Compared to population-derived cut-off limits, such as the 50% Hct limit or the 2.4% reticulocyte limit⁹, the fluctuations allowed are smaller when using individual-based cut-offs. However, a wide range of different pre-analytical factors, such as tourniquet time, body posture and the blood drawing technique, can affect concentration-derived blood parameters, such as Hct and (Hb). Also, the intra- and inter-instrument variation must be considered for all measured parameters and allowed for¹⁰⁻¹². In addition, the period from collection to analysis is critical because parameters for (Hb), Hct and %reticulocytes are relatively unstable and therefore must be analysed as quickly as possible after collection. Keeping the blood samples chilled reduces the storage-induced changes in the measured parameters, but cannot prevent fluctuations within 1-2 days¹³. In order to prevent this from occurring is to analyse the blood 'on-site'. Analysing blood 'on-site' has some major advantages. Not only is the blood fresh when it is analysed, but the result of the analysis is provided immediately, making it possible to do immediate follow-up testing if required, i.e. collecting urine samples if the blood results are suspicious. In addition, the previously mentioned refinement of the existing urine rHuEPO test has made it possible to differentiate exogenous from endogenous EPO in serum², and it is therefore recommended that blood used for screening is separated by centrifugation immediately after analysis and the serum stored frozen for subsequent rHuEPO analysis. This is of major importance, because the window of detection for rHuEPO, for example, can be reduced to as little as 12-18 hours when micro-dosages are administered¹⁴. Finally, the inter-instrument variation¹⁵⁻¹⁶ can be prevented if the same blood analyser is used for every analysis.

There are also different biological factors that can affect the results of the analysis. Previous strenuous exercise can lead to immediate dehydration and subsequently hemodilution, resulting in increases and decreases in concentration-derived



parameters, respectively¹⁷⁻¹⁸. Staying at altitude will result in an immediate hemoconcentration followed by an increase in total haemoglobin mass (HbMass), which will increase the Hct and (Hb). It is therefore important to adhere to strict pre-analytical and analytical protocols to reduce analytical variations, and to take these variations into consideration when new values are compared with 'historical' values. Information about such potentially confounding factors should be noted during sample collection and typed into the web-based database, together with the analysis result, making it

possible to consider these factors during interpretation. If samples are transported to a laboratory and the results are left pending for a period of time, the sample codes and additional information from the sample collection should be written into the database immediately after collection to keep track of any pending samples. To reduce the chain of custody, the laboratory should have direct access to the results database and type in the result immediately after analysis. The name of the athlete should be blinded to the laboratory.

Table 1: Blood screening results and rHuEPO urine test results from a male rider

Sample Date	Test Type	[Hb] (g/dL)	Hct (%)	%rets (%)	OFF-hr score (Points)	Hb z-score (SD)	OFF-hr z-score (SD)	BAP (%)
Dec. 2007	Blood	14.5	43.7	0.84	90.0			
Jan. 2008	Blood	14.7	43.0	0.50	104.6	0.2	0.84	
Jan. 2008	Urine							9
Feb. 2008	Blood	14.9	45.2	1.37	78.8	0.3	-1.24	
Apr. 2008	Blood	14.5	43.2	1.55	70.3	-0.2	-1.48	
May 2008	Blood	14.2	44.7	0.88	85.7	-0.6	-0.01	
May 2008	Blood	14.1	43.9	1.07	78.9	-0.6	-0.52	
June 2008	Blood	15.5	47.5	1.20	89.3	1.3	0.35	
June 2008	Urine							82
July 2008	Blood	14.3	44.0	0.33	108.5	-0.4	1.78	
July 2008	Blood	15.3	47.4	0.42	114.1	1.0	2.00	
July 2008	Urine							16

Blood, blood sample analysed for haemoglobin concentration (Hb), hematocrit (Hct), and percentage of reticulocytes (%rets). OFF-hr score, stimulation index calculated from the (Hb) and %rets; Hb z-score, statistical longitudinal approach of examining changes in (Hb)⁷; OFF-hr z-score, statistical longitudinal approach of examining changes in OFF-hr score⁷. Urine, urine sample analysed for recombinant Human Erythropoietin (rHuEPO) by is electric focusing (IEF) and double blotting; BAP, Basic Area Percentage is the sum of the relative intensities of bands 1-6 in the basic area of the image. A BAP > 80 % was previously used by WADA as a criterion for positivity¹⁹.

A stronger collaboration between laboratories and testing officials is essential in optimising the current anti-doping work. For example, quantitative data from the urine rHuEPO test, which are currently not provided to the testing officials, possess valuable information of potential doping abuse, and can be used in a longitudinal fashion alone or in tandem with blood screening results if provided. Table 1 exemplifies this approach, where longitudinal blood screening results and longitudinal quantitative data from the urine rHuEPO test from the same athlete are used together in the interpretation. The %rets levels of 0.33 and 0.42 are not only considered 'suspicious'

because of their absolute values (normal range 0.5-1.5) but also because they are markedly lower than previously measured values (Table 1). Such a decrease is compatible with previous rHuEPO administration. This hypothesis is supported by the quantitative data from the rHuEPO, where a high basic area percentage (BAP) (82 %) is compatible with current rHuEPO abuse (previously, a BAP >80% was used to determine a test result as an adverse analytical finding¹⁹, which was observed a few weeks before the low %rets were measured, and where the blood profile in general was considered normal. Such data should be made available to the testing body



and typed directly into the database by the laboratory, thereby providing as much relevant information from the analysis as possible. Subsequently, test results should be made available and interpreted by an independent panel of experts.

Summary

A generally accepted and vital tool to guide the behaviour of a population in a certain (politically acceptable) direction is prevention. These authors believe that informing riders about the applied anti-doping programme and about doping in general, combined with proper test management, are two key ways to prevent riders from using banned substances. Therefore at convenient times team-based anti-doping lectures should be offered. The primary goal of an intensified information campaign is to demystify current and future anti-doping activities. The riders could take part in the construction of their own profiles and in the review of their test results, gaining an understanding of why tests may be positive and why medical indications of manipulations can be strong without the approval of official anti-doping authorities. In addition, suggestions from riders for new or altered anti-doping activities should be heard and implemented if relevant. These authors believe that the risk of educating the riders to better circumvent doping infractions are speculative. On the contrary, by being open-minded about the anti-doping programme - its limits and advantages – the riders would gain confidence in the applied anti-doping programme, understanding the sometimes brutal timing of collections, why small fluctuations in their profiles may be considered suspicious and, vice versa, and how large fluctuations are sometimes considered normal. In addition, publication of an annual or real-time individual's blood and urine profiles (although anonymous) would lead to an increased transparency and accountability of the programme (and the responsible anti-doping bodies), thereby increasing the trust of the riders.

Conclusions

Five doping cases during the recent Tour de France (2008) revealed an ongoing doping abuse in cycling. Although an increased number of tests and the introduction of individually-based cut-off limits decreases the room for manipulation, it is important to

intelligently schedule the sample collection, adhere to strict analytical practices to assure a high quality of the samples collected and increase the amount of data from the analysis reported by the laboratories to most efficiently combat doping in cycling. This can only be achieved by a coordinated and transparent effort, and improved collaboration between the different testing bodies, DCOs, laboratories and riders.

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References

1. Catlin DH, Fitch KD, Ljungqvist A. Medicine and science in the fight against doping in sport. *J Intern Med* 2008; 264(2): 99-114.
2. Lasne F, Martin L, Martin JA, et al. Isoelectric profiles of human erythropoietin are different in serum and urine. *Int J Biol Macromol* 2007; 41(3): 354-357.
3. Lasne F, Martin L, Crepin N, et al. Detection of isoelectric profiles of erythropoietin in urine: Differentiation of natural and administered recombinant hormones. *Anal Biochem* 2002; 311(2):119-126
4. Birkeland KI, Stary-Gundersen J, Hemmersbach P, et al. Effect of RhEPO administration on serum levels of STfR and cycling performance. *Med Sci Sports Exerc* 2000; 32(7):1238-1243.
5. WADA. International Standard for Testing 2009. Available at URL: http://www.wada-ama.org/rtecontent/document/IST_E_n_2009.pdf
6. Lamon S, Robinson N, Sottas PE, et al. Possible origins of undetectable EPO in urine samples. *Clin Chim Acta* 2007; 385(1-2):61-66.
7. Sharpe K, Ashenden MJ, Schumacher YO. A Third Generation approach to detect erythropoietin abuse in athletes. *Haematologica* 2006; 91(3):356-363.
8. Robinson N, Sottas PE, Mangin P, et al. Bayesian detection of abnormal haematological values to introduce a no-start rule for heterogeneous populations of athletes.



- Haematologica 2007; 92(8):1143-1144.
9. Zorzoli M. Blood monitoring in anti-doping setting. In: Schänzer W (ed.). *Recent advances in doping analysis*. Cologne: Sport und Buch Strauss, 2005, pp.255-264.
 10. Lippi G, Bassi A, Solero GP, et al. Prevalence and type of preanalytical errors on inpatient samples referred for complete blood count. *Clin Lab* 2007; 53(9-12):555-556.
 11. Lippi G, Salvagno GL, Solero GP, et al. The influence of the tourniquet time on hematological testing for antidoping purposes. *Int J Sports Med* 2006; 27(5): 359-362.
 12. Lippi G, Salvagno GL, Solero GP, et al. Stability of blood cell counts, hematologic parameters and reticulocytes indexes on the Advia A120 Hematologic Analyzer. *J Lab Clin Med* 2005; 146(6):333-340.
 13. Robinson N, Mangin P, Saugy M. Time and temperature dependent changes in red blood cell analytes used for testing recombinant erythropoietin abuse in sports. *Clin Lab* 2004; 50(5-6):317-323.
 14. Ashenden M, Varlet-Marie E, Lasne F, et al. The effects of microdose recombinant human erythropoietin regimens in athletes. *Haematologica* 2006; 91(8):1143-1144.
 15. Ashenden MJ, Sharpe K, Damsgaard R, et al. Standardization of reticulocyte values in an antidoping context. *Am J Clin Pathol* 2004; 121(6): 816-825.
 16. Robinson N, Schattenberg L, Zorzoli M, et al. Haematological analysis conducted at the departure of the Tour de France 2001. *Int J Sports Med* 2005; 26(3): 200-207.
 17. Saris WH, Senden JM, Brouns F. What is a normal red-blood cell mass for professional cyclists? *Lancet* 1998; 352(9142): 1758
 18. Morkeberg JS, Belhage B, Damsgaard R. Changes in blood values in elite cyclist. *Int J Sports Med* 2008 [Epub ahead of print]
 19. WADA. WADA TDEPO 2004. 2004. Available at [URL:http://wada-ama.org/rtecontent/document/td2004_epo_en.pdf](http://wada-ama.org/rtecontent/document/td2004_epo_en.pdf)

