MAKING THE 'INVISIBLE' 'VISIBLE': STRUGGLE OF WADA

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ABSTRACT

The current 'prohibited list' includes hundreds of substances, ranging from volatile stimulants to modified polysaccharides and glycoproteins, considering also an increasing variety of doping methods, including blood transfusions and other forms of blood doping. The period of invisibility depends on many parameters, and can range from a few days to several years; to tell the truth, new invisible substances and methods are expected to 'show up' in the future but as far as the scientific and technologic development continues to progress, and as far as the antidoping scientists continue to be involved in research projects running parallel to their routine activity, none of the few forms of doping will remain undetectable forever. The antidoping laboratories keep pace with the evolving scenario of doping substances and methods.

Key Words: Sport Doping, Antidoping Analysis, Gas-Chromatography/Mass Spectrometry and Liquid Hromatography/mass Spectrometry.

INTRODUCTION:

Armstrong, seven-time Tour de France champion & the cancer survivor, became the symbol of a new era, on the basis, largely, of an implicit understanding that someone who had almost lost his life would not take drugs. The United States Anti-Doping Agency (USADA) accuses Lance Armstrong and others with involvement in a "pervasive pattern of doping" using the prohibited substances and methods: Erythropoietin (EPO), Blood transfusions, Testosterone, Human Growth Hormone (hGH), Corticosteroids & Saline and plasma infusions. Armstrong, who has been dogged by doping allegations throughout his career, has denied doping and has never officially tested positive. At the 1999 Tour, he failed a test for a corticosteroid, but produced a doctor's note for it.

Similarly Marion Jones-Thompson, former world champion track and field athlete, and a former professional basketball player winner of five medals at the 2000 Summer Olympics in Sydney, Australia. Marion Jones was tested 160 times at meets in her career and never had one positive test.

In October 2007 she admitted that she took performance-enhancing drugs tetrahydrogestrinone ("the Clear"), as far back as the 2000 Summer Olympics. American company Bay Area Laboratory Co-Operative (BALCO) marketed tetrahydrogestrinone ("the Clear"), a then-undetected, performance-enhancing steroid developed by chemist Patrick Arnold, had supplied a number of high-profile sports stars from the United States and Europe with "the Clear" and human growth hormone for several years.

Above examples had made it clear that there is continuous cat and mouse race between the cheaters and testers. In the last four decades, the 'prohibited list' progressively expanded, being periodically updated, first by the IOC Medical Commission itself, and then by the World Anti- Doping Agency (WADA). Also, the number of testing laboratories increased, reaching the present tally of 36 laboratories, analyzing more than 2,50,000 biological samples per year. Briefly, three main periods can be identified in the race between cheaters and testers (also outlined in Table1):

1. An early age, outlined above, corresponding to the abuse of 'in-competition' drugs: most, if not all, laboratory methods for the detection of doping substances of this kind were based on gas chromatography;

2. The androgenic anabolic steroids (AAS) age (including their endogenous prototype, testosterone), starting in the early 1970s, in which the administration of the performance-enhancing drugs took place mainly in periods of training and not (only) 'in competition'; this era also signs the development of specific analytical methods based on gas-chromatography/mass-spectrometry and, later on, on liquid-chromatography–mass spectrometry, also in theirmore advanced configurations (GC/high resolution mass spectrometry (HRMS), GC/MS-MS, GC/ isotope ratio mass spectrometry (IRMS), LC/MS-MS);

3. The protein chemistry and molecular biology age, which follows the application of routine techniques in molecular biology and genetic engineering by the pharmaceutical companies, with the production (and abuse in sport doping) of peptide hormones: analytical techniques extended beyond the borders of chromatography/spectrometry, including immunological techniques and, in general, all those analytical approaches that are generally typical of a laboratory of protein chemistry. Chronologically, this period also includes the recourse to 'blood doping', i.e. blood transfusions and administration of substances capable of increasing the capacity of oxygen transport to the muscle.

In addition to the above, a fourth period is feared by many as the next step in the illicit search for the 'better' performance-enhancing drugs and methods: the gene doping age. It is expected, however, that gene doping will not develop before gene therapy will be practically available.

Table – 1

Chronological evolution of the main challenges and solutions in doping control analysis

Period	Challenge	Solution/Testing Techniques
Origin early 1970s	Stimulants, narcotics, drugs of abuse	GC/NPD
Mid 1970s	Synthetic anabolic androgenic steroids (AAS)	GC/MS
1980–1990	Beta-blockers, diuretics, cannabinoids, Glucocorticoids	GC/MS, immunoanalysis (for screening only)
Early to mid-1990s	Low concentration of AAS by GC/HRMS	High sensitivity GC/MS techniques (e.g. GC/HRMS, GC/MS-MS)
Mid- to Late 1990s	Human chorionic gonadotropin Endogenous Testosterone and/or precursors	Immunoanalysis (mainly ELISA) GC/IRMS, also in combination with longitudinal profiling
Mid-1990–2000	Erythropoietin and analogs	Isoelectrofocusing with double blotting.
2000–2005	Designer steroids, Hormone and hormone receptors modulators	LC/MS and LC/MS-MS GC/MS and LC/MS
2003–present	Blood doping	Direct tests for hemoglobin-based oxygen carriers (HBOCs) and for homologous blood transfusions; indirect tests (e.g. longitudinal profiling, 'biological passport') for other forms of blood doping.

2005–present	Peptide hormones	Advanced LC/MS-MS techniques, used in combination with molecular biology and protein chemistry technologies.
2008–.present	Gene doping	Longitudinal profiling (‘biological passport’), advanced molecular biology methods

The goal of the scientists working in the 33 WADA-accredited laboratories is to annul or at least to minimize the gap between the substances included in the list and those that can practically be detected. Significant progress in this direction has been recorded in the last few years. In general, a substance can be ‘invisible’ for the following reasons:

1. The substance is unknown, or (which is the same, from the point of view of the antidoping laboratory) its metabolism is unknown: this means that there is no known analyte at which to ‘aim’ the analysis.
2. The concentration of the substance in the considered biological matrix is below the limit of detection of the analytical method.
3. The substance is very similar or even identical to an endogenous substance, i.e. a naturally produced substance which is normally present in all the biological samples collected for the antidoping analysis.
4. The administration of a forbidden drug is ‘masked’ by the use of other substances that are not yet recognized as such and therefore not yet included in the list, in the class of ‘masking agents’.

Nonetheless, a substance cannot remain invisible forever. Antidoping research proceeds restlessly. Several milestones in the race between dopers and testers confirm such a trend. Synthetic testosterone was ‘invisible’, and its abuse could only be proved indirectly, by longitudinal tests, until a method based on the ratio between the two stable carbon isotopes was developed and routinely applied in antidoping analysis. Increasing attention is also being devoted to the potential use of masking agents and methods, whose ‘detection’ should also involve extra laboratory resources, especially if the masking strategy (e.g. catheterization, addition of sufficient amounts of proteases to

the urine samples) is activated inside the doping control station, that is at the moment of production/collection of the sample.

ENFORCEMENT OF ANTI-DOPING POLICY TODAY:

Anti-doping is enforced by a combination of repression and surveillance. The latter includes the so-called 'whereabouts' rule, or the obligation for a selected pool of elite athletes to inform the anti-doping authorities where they will be each day of the year, to allow unannounced out-of (and in)-competition testing, with the obligation to be present at the announced site for one specific hour per day. The athletes have to provide this information to the authorities in advance, four times a year for three months periods at a time, using electronic and paper-based means and informing in time of any changes. This rule aims at preventing out-of-competition doping in preparation for competition. To force athletes to comply, three missed tests within an 18-months period constitute a doping offence. The actual testing involves providing urine samples (produced in full view by an anti-doping officer), consenting to blood sampling, and in some instances also providing hair samples for doping history and tissue for gene profiling for forensic practices.

Longitudinal testing, looking for fluctuations in certain blood parameters compatible with doping, is now also being introduced. This practice, known as the 'athlete biological passport' (ABP), has recently led to the first indictments of athletes, based on indirect indices of presumed doping rather than laboratory tests directly showing the presence of the forbidden substances or their metabolites in urine or blood. The authorities see the ABP as an improvement of anti-doping. But the ABP may produce false-positive results due to analytical variability and outlying individual patterns resulting from the effects of behaviour (training, altitude exposure) and genetics.

Mostly related to its enforcement strategies, anti-doping has a non-negligible cost. The IOC finances half the budget of the WADA, while the other half comes from national governments. National anti-doping agencies are mostly co-financed by national sports federations and governments. Overall the tendency is towards increasing costs with a new costly anti-doping industry steadily asking for more. The application of new national anti-doping legislations also comes with an increase in cost. Taken together, all of these costly surveillance practices seriously impinge upon the privacy of athletes and set them apart from the general population, for whom the

protection of the private sphere and autonomy are generally respected in democratic societies, and are at odds with general relaxed attitudes of modern society towards human enhancement practices.

CONCLUSION:

Anti-doping is aiming for a similar unattainable goal, sports without doping and has adopted the —"Just say no"— slogan from the 'war on drugs' movement. The sports movement has created a utopian vision of what a human should aspire to, a young beautiful athlete with perfect behaviour. This vision is used to lever unprecedented means to combat drugs in sports and now also outside sports, that put into question all what the harm reduction movement has fought for. A final observation deals with the health risks of doping substances and methods: to protect the athletes means, first of all, to protect their health. This cannot be achieved simply by the antidoping tests, but by promoting the culture of a doping-free sport and circulating information on the many potential health risks consequent to the misuse of substances/methods included in the 'prohibited list.' A deeper knowledge of the actual risks of the abuse of doping substances and methods should be promoted, going beyond the limits imposed by the current forensic approach, and considering the toxicological relevance not only of 'markers of exposure' but also of 'markers of effect' of doping substances and methods.

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