Performance-Enhancing Drugs I: Understanding the Basics of Testing for Banned Substances

Amy B. Cadwallader and Bob Murray

Whenever athletes willfully or accidentally ingest performance-enhancing drugs or other banned substances (such as drugs of abuse), markers of those drugs can be detected in biological samples (e.g., biofluids: urine, saliva, blood); in the case of some drugs, that evidence can be apparent for many weeks following the last exposure to the drug. In addition to the willful use of prohibited drugs, athletes can accidentally ingest banned substances in contaminated dietary supplements or foods and inadvertently fail a drug test that could mean the end of an athletic career and the loss of a good reputation. The proliferation of performance-enhancing drugs and methods has required a corresponding increase in the analytical tools and methods required to identify the presence of banned substances in biofluids. Even though extraordinary steps have been taken by organizations such as the World Anti-Doping Agency to limit the use of prohibited substances and methods by athletes willing to cheat, it is apparent that some athletes continue to avoid detection by using alternative doping regimens or taking advantage of the limitations in testing methodologies. This article reviews the testing standards and analytical techniques underlying the procedures used to identify banned substances in biological samples, setting the stage for future summaries of the testing required to establish the use of steroids, stimulants, diuretics, and other prohibited substances.

Keywords: anti-doping; prohibited substances; contaminated supplements

This is the first in a series of articles on performance-enhancing drugs, including the topic of contaminated dietary supplements, intended to highlight the challenges associated with verifying the use of substances and methods designed to give athletes an unfair competitive advantage.

Athletes who decide to cheat can choose from a large arsenal of performance-enhancing drugs (PEDs) that include anabolic agents, stimulants, peptide hormones and growth factors, aromatase inhibitors, and diuretics, as well as illegal methods such as blood doping, sample contamination, and intravenous infusions. Athletes can also inadvertently ingest banned substances in over-the-counter drugs (OTCs), in dietary supplements, from contamination of prescriptions from pharmacies, or even in the food they eat (Brown, 2014; H. Geyer et al., 2014; Thevis et al., 2013). In fact, some dietary supplements include banned substances that are listed among their ingredients on the product labels, while other supplements are accidentally or purposefully contaminated with unlisted banned substances or potentially dangerous ingredients (e.g., ibuprofen in sensitive individuals or prescription diuretics) during manufacturing (Cadwallader et al., 2013; Zeltner, 2014).

Drug testing (antidoping) programs can be an effective means of identifying some of those who do cheat and are hopefully effective at dissuading others from cheating. The World Anti-Doping Agency (WADA; www.wada-ama.org) is the most recognized agency responsible for identifying prohibited substances and methods, creating guidelines for athlete testing, and establishing standards for the analyses of biological samples (urine, blood, oral fluid, etc.). The World Anti-Doping Code (The Code) is the core document that standardizes antidoping policies, rules, and regulations within many sporting organizations around the world. There are five International Standards that fall under The Code which aim to foster consistency among National Anti-Doping Organizations (NADOs) and laboratories. These International Standards include: testing; laboratories; Therapeutic Use Exemptions (TUEs); the List of Prohibited Substances and Methods; and the protection of privacy and personal information (WADA, 2015).

Several changes will take effect in The Code in 2015 (the first since 2009). Some of the more noteworthy changes applicable to the topics discussed in this review include: 1. Ineligibility for real cheats and more flexibility and leniency in sanctioning other specific circumstances (i.e., those that intentionally take PEDs can be barred from competition for 4 years and those who can prove no fault after an adverse analytical finding can receive more...
lenient sanctions); 2. Intelligence in the fight against doping is deemed more important (e.g., investigations and obtained evidence in the fight against doping will be highlighted in addition to adverse analytical findings); 3. Athlete support personnel will be held more accountable for their role in aiding doping (WADA, 2014, 2015).

The WADA Anti-Doping Code (WADA, 2015) defines the use of performance-enhancing drugs and methods as “contrary to the spirit of sport.” Additionally, in The Code WADA lists three criteria for consideration when reviewing substances of methods for inclusion on The Prohibited List. Any substance or method added to The Prohibited List must fulfill at least 2 of the following: 1. Potential to enhance or proof of enhancing sport performance; 2. Evidence of a potential or actual health risk to an athlete; 3. Use violates the spirit of sport as described in The Code (Mazzoni et al., 2011; WADA, 2015).

There are roughly three dozen WADA-accredited drug-testing laboratories worldwide (as well as some independent laboratories) that process samples collected both in- and out-of-competition. Each year, WADA updates its list of prohibited substances and methods (Mazzoni et al., 2011; WADA, 2013a) to provide athletes, coaches, and support staff a comprehensive list of prohibited drugs and practices (see Table 1.) WADA also maintains a Monitoring Program for stimulants (including caffeine and synephrine; in-competition only), narcotics (such as hydrocodone and tramadol; in-competition only), and glucocorticosteroids (out-of-competition only) as a means of tracking patterns of misuse among athletes.

### Table 1 The WADA 2014 Prohibited List

<table>
<thead>
<tr>
<th>Prohibited Class</th>
<th>Examples</th>
<th>Additional Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonapproved Substances</td>
<td>Designer drugs, preclinical drugs, discontinued drugs, veterinary drugs</td>
<td>Any pharmacological substance not included in the categories below and with no current approval as a therapeutic agent</td>
</tr>
<tr>
<td>Anabolic Agents</td>
<td>Exogenous Steroids</td>
<td>Not naturally produced by the body</td>
</tr>
<tr>
<td></td>
<td>Boldenone, methylnandrol, and stanozolol</td>
<td></td>
</tr>
<tr>
<td>Endogenous Steroids</td>
<td>Androstenedione (Andro), epitestosterone, and dihydroepiandrosterone (DHEA)</td>
<td>Naturally produced by the body but administered exogenously</td>
</tr>
<tr>
<td>Other Anabolic Agents</td>
<td>Clenbuterol, androgen receptor modulators (SARMs), and zilparterol</td>
<td></td>
</tr>
<tr>
<td>Peptide Hormones, Growth Factors, and Related Substances</td>
<td>Erythropoiesis-Stimulating Agents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erythropoietin (EPO), darbepoietin (dEPO), and methoxy polyethylene glycol-epoetin beta (CERA)</td>
<td>Only in males</td>
</tr>
<tr>
<td></td>
<td>Chorionic Gonadotrophin (CG or HCG) and Luteinizing Hormone (LH) and their releasing factors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corticotrophins and their releasing factors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corticotropin-releasing factor (CRP) and adrenocorticotrophic hormone (ACTH)</td>
<td></td>
</tr>
<tr>
<td>Other Growth Factors</td>
<td>Fibroblast growth factors (FGFs), mechano growth factors (MGFs), and vascular-endothelial growth factors (VEGF)</td>
<td></td>
</tr>
<tr>
<td>Beta-2 Agonists</td>
<td>Clenbuterol, metaproterenol</td>
<td>Except inhaled asthma medications such as salbutamol, formoterol, and salmeterol. These medications require a Therapeutic Use Exemption (TUE) and are subject to threshold limits in urine.</td>
</tr>
</tbody>
</table>

(continued)
### Table 1 (continued)

<table>
<thead>
<tr>
<th>Prohibited Class</th>
<th>Examples</th>
<th>Additional Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hormone and Metabolic Modulators</strong></td>
<td>Aromatase inhibitors</td>
<td>Anastrozole, formestane, and testolactone</td>
</tr>
<tr>
<td></td>
<td>Selective estrogen receptor modulators (SERMs)</td>
<td>Tamoxifen</td>
</tr>
<tr>
<td></td>
<td>Other antiestrogenic substances</td>
<td>Fulvestrant</td>
</tr>
<tr>
<td></td>
<td>Agents modifying myostatin functions</td>
<td>Myostatin inhibitors</td>
</tr>
<tr>
<td></td>
<td>Metabolic modulators</td>
<td>Insulin and peroxisome proliferator activated receptor (PPARs) agonists</td>
</tr>
<tr>
<td><strong>Diuretics and Other Masking Agents</strong></td>
<td></td>
<td>Acetazolamide, desmopressin, glycerol, albumin, and furosemide</td>
</tr>
<tr>
<td><strong>Manipulation of Blood and Blood Components</strong></td>
<td></td>
<td>Blood doping and artificial hemoglobin</td>
</tr>
<tr>
<td><strong>Chemical and Physical Manipulation</strong></td>
<td></td>
<td>Tampering with biological samples, urine substitution, and intravenous infusions of more than 50 ml/6 hr, except during hospital admissions</td>
</tr>
<tr>
<td><strong>Gene Doping</strong></td>
<td></td>
<td>The transfer of nucleic acids and the use of normal or modified cells</td>
</tr>
</tbody>
</table>

### Prohibited In-Competition

<table>
<thead>
<tr>
<th>Prohibited Class</th>
<th>Examples</th>
<th>Additional Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stimulants</strong></td>
<td>Non-Specified Stimulants</td>
<td>Amphetamine, cocaine, and fenethylline</td>
</tr>
<tr>
<td></td>
<td>Specified Stimulants</td>
<td>Cathine, cathinones, ephedrine, epinephrine, methamphetamine, methylhexaneamine (DMAA), pseudoephedrine, and strychnine</td>
</tr>
<tr>
<td><strong>Narcotics</strong></td>
<td></td>
<td>Buprenorphine, fentanyl, methadone, morphine, and oxycodone</td>
</tr>
<tr>
<td><strong>Cannabinoids</strong></td>
<td></td>
<td>Natural and synthetic</td>
</tr>
<tr>
<td><strong>Glucocorticosteroids</strong></td>
<td></td>
<td>Cortisol, cortisone, prednisone, and aldosterone</td>
</tr>
<tr>
<td><strong>Alcohol</strong></td>
<td></td>
<td>Only in certain sports such as darts, shooting, and some ski events</td>
</tr>
<tr>
<td><strong>Beta-Blockers</strong></td>
<td></td>
<td>Atenolol, propanolol, and timolol</td>
</tr>
</tbody>
</table>

### Monitoring Program

<table>
<thead>
<tr>
<th>Prohibited Class</th>
<th>Examples</th>
<th>Additional Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stimulants</strong></td>
<td></td>
<td>Bupropion, caffeine, nicotine, phenylephrine, phenylpropanolamine, pipradrol, pseudoephedrine (&lt;150 mg/mL), synephrine</td>
</tr>
<tr>
<td><strong>Narcotics</strong></td>
<td></td>
<td>Hydrocodone, mitragynine, morphine/codeine ratio, tapentadol, tramadol</td>
</tr>
<tr>
<td><strong>Glucocorticosteroids</strong></td>
<td></td>
<td>Cortisol, cortisone, prednisone, and aldosterone</td>
</tr>
</tbody>
</table>

*Note: This is not a comprehensive list of ALL WADA prohibited substances, but a representative listing of examples. For a complete listing of substances and methods, please see The Prohibited List (WADA, 2013a).*
WADA holds athletes to a policy of strict liability, meaning that an athlete is responsible for the substances in his or her body and the subsequent consequences if metabolites or markers are found in a biofluid (typically urine, blood, or oral fluid in the context of sports doping testing)—whether the athlete intentionally or inadvertently ingested a prohibited substance (e.g., in a contaminated dietary supplement). Regardless of intent, the athlete risks an antidoping rule violation and being suspended or banned from competition. The provision of strict liability is also a common feature of many drug testing programs not affiliated with WADA.

Other sports organizations that are not affiliated with the International Olympic Committee (IOC) or WADA maintain their own banned substance lists, although most are similar in many respects to the WADA Prohibited List, which is considered the International Standard. Understandably, high schools, colleges, and other sports organizations may choose to test for a subset of WADA’s list of prohibited substances simply due to cost considerations.

PED use can be prohibited by sports governing bodies (SGBs) in-competition, out-of-competition, or just in certain sports (e.g., alcohol and beta-blocker use in shooting sports.) Each organization or SGB (that is not affiliated with WADA) establishes guidelines that determine how many athletes will be tested, when the testing will occur, and the substances to be tested. This is in contrast to WADA-affiliated organizations (NADOs) which establish the number of tests allocated to a sport and which athletes to test. NADOs must adhere to the requirements documented in The International Standard for Testing which resides under the WADA code (WADA, 2012, 2015); independent SGBs are free to create their own policies. No matter the number of tests performed or the number of athletes tested, the core tests performed and the analytical methods used (and discussed here and in subsequent papers) typically remain the same.

**History of PED Testing**

The IOC instituted a formal drug-testing program for the 1968 Olympic Games in Mexico City because it had become apparent that some athletes were using stimulants and other drugs to create an unfair competitive advantage. The analytical technique of gas chromatography-mass spectrometry (GC-MS) was used for the first time at the 1972 Munich Olympics. The combined screening-and-confirmation paradigm commonly used today was used for the first time during the Montreal Olympic Games in 1976 (Bowers, 2009). As rumors of drug use by athletes continued, drug testing programs have steadily increased in size and scope. This continual expansion in testing has required a commensurate expansion in analytical equipment and techniques.

PED testing programs can vary widely in the details of when and how athletes are tested, the substances tested, and the penalties associated with positive results. It is helpful to keep in mind that, in contrast to WADA’s testing program for athletes, other drug testing programs and SGBs involving athletes are more the equivalent of workplace drug testing commonly used by employers either as a prerequisite for hiring or conducted randomly at the workplace. Employers determine the scope and frequency of drug testing, along with the penalties associated with positive tests. For example, professional athletes are employees of large business enterprises such as the National Football League, Major League Baseball, and NASCAR (and similar sports businesses in other countries). Consequently, those organizations (businesses) determine and administer their own drug testing policies. Policies also vary among amateur sports. The National Collegiate Athletic Association (NCAA), as one high-profile U.S. example, mandates drug testing during NCAA championship events, allowing individual NCAA colleges and universities to establish and administer their own drug testing programs during regular season sporting events. The NCAA, can however, perform random tests of NCAA athletes at any college or university during the regular season. The same is true for other sports organizations that do not have to abide by IOC/WADA or other mandates.

Penalties for positive results also vary widely from sports organization to organization—from issuing reprimands, to requiring athletes to enter treatment programs (while still competing), to suspensions of varying durations, to being banned from competition for life. Although the penalties do vary significantly from organizations to SGB to NADO, keep in mind that the fundamentals of PED testing remain the same.

**Fundamentals of PED Testing**

The collection of biological samples (e.g., urine, blood, oral fluid) from athletes typically follows strict protocols that have been established to protect the rights of athletes and ensure the security and integrity of the samples. Urine is the most common biological sample collected, although blood samples are needed to test for evidence of blood doping or transfusions. Blood has to be collected and transported under special conditions (with regard to temperature) and analyzed within a strict time frame. Oral fluid is noninvasive and easy sample to collect and is increasing in popularity among SGBs.

Once a urine sample has been collected, the sample is split in the presence of the athlete into two containers, creating an A specimen and a B specimen. The A specimen is tested and the B specimen is stored and referenced if the A specimen tests positive. When positive test results do occur from the A sample, the sealed B specimen is available for analysis. The B specimen is tested only at the request of the athlete or a designated representative, sometimes in a different laboratory (depending upon the rules of the sports organization) and witnessed by observers that have been approved by the athlete. Chain-of-custody protocols have been established to ensure that biological samples are correctly collected, handled, transported, and analyzed to eliminate the risk of
altering the sample. In addition, drug testing laboratories often attain a variety of state and federal licenses and accreditations to ensure valid results.

Athlete urine samples are often tested immediately upon collection for specific gravity, creatinine, and pH as measures of specimen validity and to ensure that the sample has not been altered. Because the athlete’s hydration status affects urine specific gravity, cut-off values have been created and normalization procedures have been developed to prevent the results from being skewed by extremely dilute or concentrated urine (Crouch & Shelby, 2013).

In the drug testing laboratory, an aliquot of the urine from the A specimen is prepared for analysis and subjected to a screening assay to determine if there is evidence of PEDs or their metabolites in the urine. If the screening returns a nonnegative result (indicating the possibility of a banned substance), confirmation testing is conducted to confirm or reject the result of the screening test. The type of testing used in the screening and confirmation procedures depends upon the substances being assayed. For example, peptide hormones are screened and confirmed using immunoassay techniques whereas stimulant screening and confirmation is typically conducted using chromatography (gas or liquid; GC or LC) and mass-spectrometry (MS) procedures. If the screening procedure returns a nonnegative result, then another aliquot of the A specimen is sent through confirmation testing; screening procedures are usually qualitative testing while confirmation tests are typically quantitative. In simple terms, chromatography is used to separate the various compounds in the urine specimen before injecting into a mass spectrometer for identification of the compounds and their concentrations (Jenkins, 2013). In future articles dealing with specific categories of PEDs (e.g., endogenous and exogenous steroids, stimulants, etc.), more details about the strengths and limitations of drug testing methodologies will be reviewed.

To ensure the accuracy and reliability of the screening and confirmation techniques, quality-control procedures are consistently used. For example, testing the A specimen is often accompanied by tests of a drug-free urine specimen (negative control), one or more calibrators, and quality-control (QC) specimens that contain varying concentrations of the drugs to be measured to ensure the instrumentation is able to accurately measure at low and high concentrations (Crouch & Shelby, 2013). The calibrators and QC specimens are critical to the accurate identification and measurement of the drugs in biological specimens and are made from certified reference materials that allow traceability to the International System of Units (SI) (Mackay & Kazlauskas, 2011).

Some sports organizations, as well as WADA, have established threshold limits for some PEDs. As an example, WADA established a threshold limit of 150 micrograms per milliliter (mcg/mL; 150 ppm) for pseudoephedrine, a stimulant that is commonly found in OTC medications (WADA, 2013a). In other words, a positive result for pseudoephedrine occurs when its concentration in urine exceeds the threshold limit of 150 mcg/mL. This standard applies to in-competition testing only.

More details about the specifics and applications of blood and oral-fluid testing will be discussed in future articles.

**Fundamentals of Testing for PED Contaminants in Dietary Supplements**

When an athlete tests positive for a PED, a common excuse is that the athlete must have consumed a dietary supplement that was contaminated with a banned substance. In fact, this assertion could be true because there is evidence that supplements can be contaminated with PEDs (Cohen, 2009; Maughan, 2004; Van Thuyne et al., 2006). In such cases, if the athlete can produce the supplement in question, that supplement, along with other sealed samples from the same supplement production lot, can possibly be tested for the presence of banned substances. It should be noted that the testing of some supplements requires a substantial amount of the original product that was ingested, an amount that may no longer be available. In addition, because of strict liability, even if a supplement is found to be contaminated, this knowledge does not relieve the athlete of responsibility for the positive test, although the results may be taken into consideration in determining the appropriate penalty.

Consumers (athletes) expect the list of ingredients on the supplement label to be accurate and complete; however, cases of supplement contamination, either intentional or not, are well documented. From the period January 2004 through December 2012, 51% of all drug recalls in the United States were classified as dietary supplements (as opposed to pharmaceuticals) (Harel, Harel, Wald, et al., 2013). Several investigators have published reports detailing the detection of contaminants in dietary supplements (Van Thuyne et al., 2006). The contaminants in the products include stimulants (Cohen et al., 2013; H. Geyer et al., 2008), estrogenic compounds (Monika Plotan et al., 2014), prescription diuretics (Cadwallader et al., 2013; Hoggan et al., 2007), and anabolic agents (Geyer et al., 2003; Geyer et al., 2004; Parr, Fussholler, et al., 2011; Parr et al., 2007; Parr et al., 2009; Parr, Opfermann, et al., 2011; van der Merwe & Grobbelaar, 2005), including anabolic-androgenic steroids (AAS), designer steroids, and prohormones. Many anabolic agents found in contaminated dietary supplements are not explicitly mentioned on banned substance lists, but are alluded to by the wording “and related substances with a similar chemical structure or similar biological effect(s).”

In 1994, the U.S. Congress passed the Dietary Supplement Health and Education Act (DSHEA) (“Dietary Supplement Health and Education Act of 1994,” 1994) establishing regulations for the dietary supplement industry, effectively creating a subcategory...
of regulations related to conventional food/beverage products with oversight by the U.S. Food and Drug Administration (FDA). DSHEA defined a dietary supplement as a vitamin, mineral, herb or other botanical, amino acid, dietary substance used to increase total dietary intake, or a concentrate, metabolite, constituent, extract, or combination of ingredients such as enzymes, metabolites, organ tissues, and glandulars. It also states that dietary supplements should be used in combination with a “healthy diet.” DSHEA also established go-to-market guidelines that enable supplement manufacturers to include any ingredients that were marketed before October 15, 1994 without prior approval from the FDA. Manufacturers are responsible for notifying the FDA if they intend to use new ingredients and provide evidence of ingredient safety. Although the FDA oversees dietary supplements, it does not approve supplements for safety or effectiveness. Manufacturers are responsible for assuring their products are safe and no proof of product effectiveness is required before dietary supplements are sold to the public. In addition, the FDA has to demonstrate that a supplement is unsafe before taking action to limit use of the product or mandate its removal from the marketplace (usually relying on a compilation of adverse-event reports from physicians and consumers). If dietary supplements are found to be contaminated with unsafe ingredients or prescription drugs, the FDA can issue fines and require that the contaminated products be removed from store shelves and destroyed. However, manufacturers are usually free to reintroduce a reformulated version of the product.

AAS were added to the U.S. Federal Schedule of Controlled Substances in 1990. With the passing of DSHEA in 1994, the sale of steroid precursors as “food supplements” was legal. Some steroid precursors were subsequently included in banned lists, but many were not and supplement manufacturers continue to take advantage of this loophole in the laws and regulations. Even more stringent regulations arrived with the approval of the U.S.’s Anabolic Steroid Control Act of 2004 (“Anabolic Steroid Control Act of 2004,” 2004). The intent of these regulations was to limit the availability and discourage the use of AAS, but they inadvertently encouraged the clandestine synthesis of structurally unique steroids and the addition of prohormones to dietary supplements. These prohormones and designer steroids are produced to avoid the regulations and to evade detection and identification by antidoping laboratories. Minor structural modifications of an existing steroid can increase its anabolic potency and make it resistant to metabolism. In addition, these designer compounds are manufactured to resemble existing steroids, but with enough modification to evade detection by antidoping laboratories (Kazlauskas, 2010). Many of these compounds are developed from historical publications (Vida, 1969) and are added to dietary supplement products without efficacy, safety, or toxicity assessments. Recently, the Designer Anabolic Steroid Control Act of 2014 (“Designer Anabolic Steroid Control Act of 2014,” 2014) was introduced to the U.S. Congress. This bill aims to close the loophole in the Anabolic Steroid Control Act of 2004 and give authorities more power in punishing those who develop unique steroidal compounds. Considerably more detail will be provided on these topics in future articles.

Contamination of dietary supplements can be purposeful or accidental: the latter resulting from sloppy manufacturing processes, including poor quality control/assurance of ingredient suppliers, and the former the result of unscrupulous and sometimes criminal behavior on the part of manufacturers. Muscle-building, weight-loss, and sexual-enhancement supplements have the greatest incidence of contamination with steroids, stimulants and diuretics, and erectogenics, respectively (Cohen, 2009; H. Geyer et al., 2008; Harel, Harel, & Bell, 2013; Maughan, 2004; van der Merwe & Grobbelaar, 2005; Van Thuyne et al., 2006). Herbal products have also been identified as high risk. In addition, supplement products do not always include the active ingredient contents they claim (Van Thuyne et al., 2006). For example, the label might claim to have 100 mg of an active ingredient but actually contain only 20 mg or even 200 mg of the ingredient.

Testing of dietary supplements for PED contaminants follows a similar methodology as testing of urine specimens, using LC/MS and GC/MS techniques to isolate and identify the compounds of interest. One laboratory challenge in assaying supplements is in developing reliable methods for extracting PEDs from the different supplement matrices—tablets, capsules, powders, gels, bars, beverages, etc. Once those methodologies are in place, testing laboratories can identify the presence of PEDs, at concentrations that are often dependent on the matrix of the dietary supplement (but frequently in concentrations less than 5 parts per billion (ppb)).

Even the most sophisticated analytical testing techniques can miss PED contaminants in urine and supplements simply because the techniques “look” for certain PEDs and not others. For this reason, designer drugs such as the steroid tetrahydrogestrinone (THG; “the clear”) (Malvey & Armsey, 2005), along with discontinued, unapproved, or analog drugs can escape detection until GC/MS or LC/MS/MS techniques are developed to identify those compounds. Most illicit compounds can eventually be detected once scientists know what chemical structure to include in testing. The unfortunate cat-and-mouse game that is now part of PED testing is created by the development of new PEDs that escape initial detection.

In addition, other innovative methodologies are currently used for supplement testing. Yeast and mammalian cell-based bioassays, which can detect both the presence and relative bioactivities of substances in a sample, are employed to detect designer steroids, prohormones, and estrogenic compounds present in dietary supplements (Akram et al., 2011; Cadwallader et al., 2011; Cadwallader et al., 2012; Cooper et al., 2013; Houtman et al., 2009; Monika Plotan et al., 2014; M. Plotan et al., 2011; Rijk et al., 2009). Biological assays are advocated because they detect one or more
biological effects of prohibited substance use (Pozo et al., 2013). These methods do not require knowledge of the compound’s structure and use biological endpoints of the drugs’ action as opposed to detecting the presence of the drug or its metabolite(s).

Bioassays, therefore, detect all compounds interacting with a chosen endpoint regardless of their structure. Bioassays using living cells represent the in vivo drug actions of receptor activation, compound uptake into cell, and eventual protein production. Other applications of bioassays, beyond their ability to simply detect the presence of banned substances, include their use in conjunction with mass spectrometers to determine the structure of new ligands, their capability to determine the relative biological activity of steroid receptor ligands, and the ability to be coupled with other molecular biology techniques such as microsomal metabolism for additional studies on newly identified compounds. With all of the benefits that bioassays provide, their use in the fight against antidoping is increasing.

PED testing of biological specimens includes parent compounds and their metabolites and analogs (e.g., metabolites and analogs of testosterone). PED testing of supplements (and nonbiological specimens) typically includes only the parent compounds, allowing unscrupulous manufacturers a loophole of sorts to include metabolites and analogs of PEDs that might confer an ergogenic effect yet avoid detection.

Supplement manufacturers can choose to have their products tested and certified by third parties with the technical aptitude to analyze supplements for banned substances. However, even when a product has gone through the necessary testing to obtain certification, it is impossible for manufacturers to claim that a product is “free of banned substances” simply because it is not possible to test for all banned substances. For example, the WADA list of prohibited substances encompasses hundreds of different compounds along with the repeated proviso that includes “other substances with a similar chemical structure or similar biological effect(s).” The most that a supplement certification process can do is to certify that the supplement is free of the banned substances that were part of the certification testing protocol. However, it is important to keep in mind that manufacturers whose products bear a reputable certification label and follow current good manufacturing practices (cGMP) have made a good-faith effort to ensure consumers that their products have been tested and that certain banned substances such as steroids, stimulants, or diuretics have not been found (Buell et al., 2013). In short, supplement certification cannot completely eliminate the risk that a supplement is contaminated, but it is strong evidence of reduced risk.

**Athlete Biological Passport (ABP)**

Longitudinal testing, also referred to as the Athlete Biological Passport (ABP) (WADA, 2013b), is a protocol designed to create a biological baseline against which subsequent test results can be compared with establish evidence of doping. There are two WADA ABP modules: the Hematological Module which detects blood manipulation (and requires a blood sample) and the Steroidal Module which detects AAS abuse (and requires a urine sample) (Vernec, 2014; Zorzoli et al., 2014). For an athlete to be a part of an ABP program, he/she must provide their daily whereabouts information to program administrators for testing at any time. Whereabouts information is submitted to NADOs through the Anti-Doping Administration & Management System (ADAMS) or via a NADO-specific whereabouts system.

On those occasions when an athlete tests positive for a low level of a PED, it may be impossible to ascertain whether the finding resulted from incidental consumption of a contaminated supplement or food, past use of higher doses of a drug, or continued use of low doses of a drug or doping method. For that reason, the ABP was established as a way to monitor athletes on an ongoing basis to provide indirect but irrefutable evidence of doping. The ABP differs from drug testing in that prohibited substances and methodologies are not the subject of testing; instead, the effects of those substances and methodologies are the subject of measurement. For example, if an athlete uses EPO or autologous blood transfusions to illicitly improve the oxygen-carrying capacity of the blood, a variety of hematological measures will change (e.g., hemoglobin, hematocrit, RBC count, percent reticulocytes, mean corpuscular volume, etc). An athlete’s initial blood or urine test serves as the baseline against which future measures are compared. Although the ABP is not foolproof, it is one more weapon in the antidoping arsenal.

**Summary**

The unfortunate reality of sports is that there will always be athletes who are willing to cheat their way to better performance, violating the very spirit of fair play and sportsmanship. Therefore, there will always be those willing to make cheating possible by creating drugs and methods designed to avoid detection. This latter group includes unscrupulous dietary supplement manufacturers who purposefully contaminate products with banned drugs and other substances; with a $60 billion dietary supplement market worldwide, the financial impetus to be unprincipled is great (Crowley & FitzGerald, 2006). Antidoping programs are designed to catch the cheaters and help ensure all athletes have a fair chance while competing. The guidelines for antidoping programs are clear and comprehensive and the testing techniques are sophisticated and precise, yet some athletes who cheat are able to avoid detection. In subsequent articles, we will overview the strengths and limitations of the testing procedures associated with endogenous and exogenous steroids, stimulants, diuretics, beta-blockers and beta-2 agonists, hormones, growth factors, and metabolic modulators.
References


