

Anabolic Steroids

Testing Athletes for Illegal Steroid Use

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Doping or steroid use is not confined to professional athletes. The International Olympic Committee administers tests for steroids and many banned substances in order to find athletes who break the rules. Colleges test their athletes, too, and now, due to increasing concerns about steroid use by high school athletes, many states' legislatures are debating whether laws should be enacted to test teens. Although programs have been put in place to educate athletes about the health risks associated with anabolic steroids, use among teens grew for years before it began to decrease slowly. According to a federally funded, annual survey conducted by researchers at the University of Michigan, 2.6 percent of twelfth graders reported in 2005 that they had taken anabolic steroids at least once, compared with 4.0 and 2.1 percent of twelfth graders surveyed in 2002 and 1991.

Athletes who use illegal steroids often show telltale signs of use, including increased aggression, mood swings, and physical symptoms—such as baldness and development of breasts in teenaged boys, and growth of facial hair and deepened voice in teenaged girls—but only a urine test can confirm suspected use.

Detecting Dopers

Athletes who take performance-enhancing steroids are secretive, although they often share drugs with buddies and take turns giving each other injections. Novices might start with pills before moving on to injectables, sometimes using different steroids simultaneously, a practice known as “stacking.” In another common practice, called “pyramiding,” athletes increase and then de-

crease doses during a multi-week regimen. The simultaneous use of other drugs—for example, human growth hormone—is also commonly employed to maximize or maintain performance enhancement. In some instances, athletes will also take drugs to counteract the steroid's side effects, such as human chorionic gonadotropin to prevent testicular atrophy or anti-estrogens to prevent gynecomastia.

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tests can choose from a myriad of methods and products available through the Internet and elsewhere. Urine substitution in males can involve a bag of clean urine under the armpit or up the colon, with tubing leading to the finger tip or penis, while females can hide urine bags in the vagina. And urine adulterants are often designed to make users drink gallons of water to eliminate the drug and dilute the urine.

custody of the samples and where they are securely stored at all times.

As shown in Figure 1, after the sample is delivered to the lab, the intact B sample is locked up, and the lab screens batches of up to 20 aliquots of the A samples. Sample preparation—including enzymatic deconjugation and chemical derivatization—takes several hours, and analysis by chromatography and mass spectrometry, the core technology in doping control labs, typically runs overnight. If a sample screens positive, a fresh aliquot of the A sample undergoes an A confirmation attempt. The lab identifies steroids in the sample by matching the results with known analytical data from an authentic reference standard. If the A confirmation proves the presence of a prohibited steroid, most sports organizations then request the B confirmation analysis (Figure 1, bottom panel).

The Anti-Doping Target List

The World Anti-Doping Agency (WADA) 2006 Prohibited List names 68 anabolic steroids in two categories: exogenous and endogenous anabolic steroids (See box, p. 13). Exogenous “refers to a substance that is not ordinarily capable of being produced by the body naturally,” such as stanozolol (Figure 2). In contrast, endogenous is defined as “a substance that is capable of being produced by the body naturally,” such as testosterone (Figure 2). Some athletes try to boost their performance by using pharmaceutical testosterone, perhaps in the form of skin patches, a practice that is banned. Other examples of pharmaceuticals misused by some athletes include: boldenone, a veterinary anabolic steroid; methandienone, a discontinued pharmaceutical formerly marketed under the brand name Dianabol; and norethone, a never-marketed pharmaceutical whose clinical trials were aborted, probably due to toxicity.

An athlete is considered to have committed a doping offense if the lab detects the presence of an exogenous anabolic steroid in the urine sample; therefore, lab testing need only be qualitative. For some endogenous steroids, WADA set cut-off levels: 19-norandrosterone (2 ng/mL); epitestosterone



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Screening Protocols

In top doping control programs, officials employ direct observation to prevent urine manipulation. The athlete must be bare from torso to knees while an official of the same sex performs a visual inspection to look for concealed devices and watches the athlete urinate. The urine sample is then split between an “A” and “B” bottle and labeled so that the lab can identify each sample only by a code number and not by the athlete's name. “Chain-of-custody” forms document

Figure 1 Typical Anti-Doping Lab Procedure

"ALiquot" (=transfer some urine)...

...THEN LOCK IN:

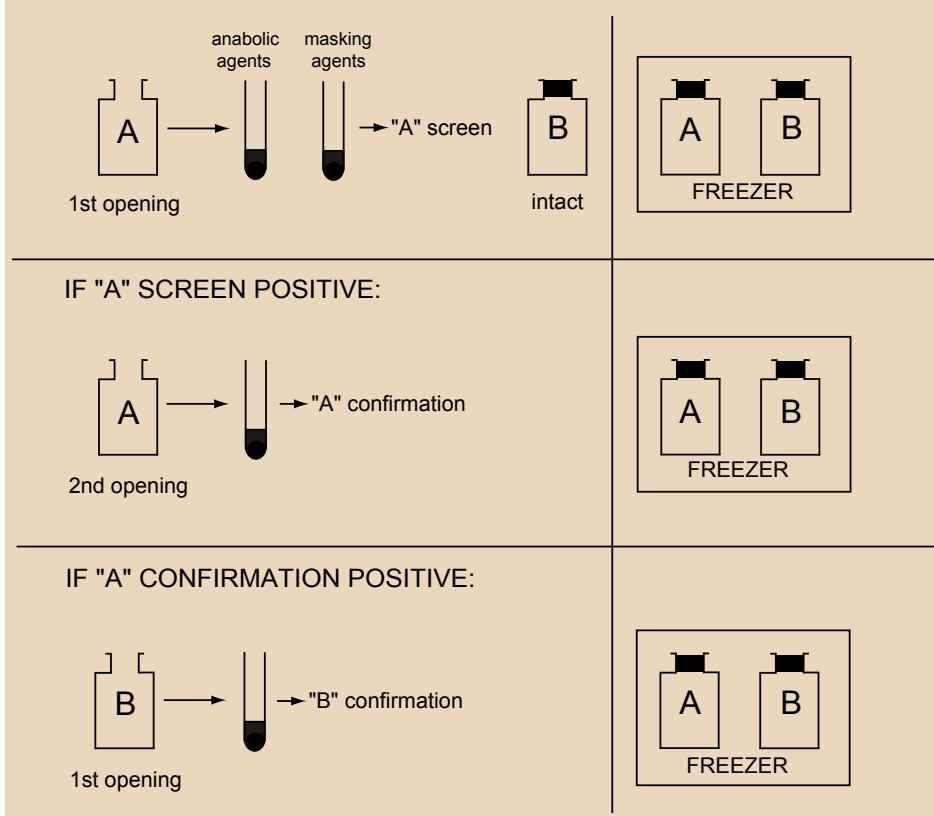
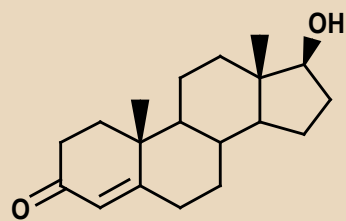
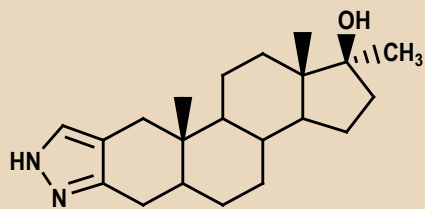


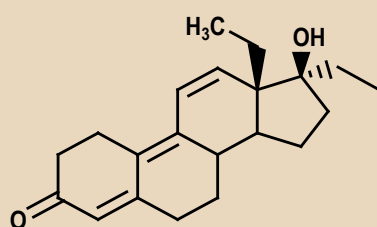
Figure 2 Chemical Structures of Anabolic Steroids Misused for Sport Doping



Testosterone



Stanozolol



Tetrahydrogestrinone (THG)

Testosterone (natural, endogenous, also available as a pharmaceutical), stanozolol (synthetic, exogenous, pharmaceutical, veterinary), and tetrahydrogestrinone or THG (synthetic, exogenous, designer steroid).

(200 ng/mL); and the urinary testosterone-to-epitestosterone ratio (T/E = 4).

As is the case for other WADA-prohibited classes of substances or methods, the list of specific examples on the list is followed by the statement: "and other substances with a similar chemical structure or similar biological effect(s)." This caveat prevents athletes from cheating simply by using an un-listed substance, particularly so-called "designer drugs" specifically created to beat detection. Because of this statement, doping control laboratories must be able to detect and identify both compounds on the prohibited list and those that have yet to make the list.

One interesting example of how complicated detecting novel designer steroids can be is the case of tetrahydrogestrinone (THG, Figure 2), the story of which occupied media headlines for many months in 2003. In this case, detection required multiple steps including: (1) elucidating the chemical formula and structure by several chromatography and mass spectrometry techniques, as well as nuclear magnetic resonance spectroscopy; (2) synthesizing a reference standard; (3) validating screening and confirmation procedures; and (4) conducting baboon excretion and metabolism studies. Throughout this process, all the steps had to be executed according to exacting scientific and legal standards.

The Technical Aspects of Steroid Testing

Unfortunately, no dipsticks, commercial kits, or immunoassays are available to test urine for anabolic steroids. While clinical tests for serum testosterone are still routinely performed by immunoassay, these assays are susceptible to cross-reactions with structurally related compounds. Not only is higher specificity necessary to confirm the presence of abused anabolic steroids, but it is also needed during screening because abused steroids must be distinguished from endogenous ones. Therefore, the methods used to test urine for anabolic steroids originate from analytical organic chemistry.

The World Anti-Doping Agency 2006 Prohibited List of Anabolic Androgenic Steroids

Exogenous

- ▶ 1-androstendiol
- ▶ 1-androstendione
- ▶ bolandiol
- ▶ bolasterone
- ▶ boldenone
- ▶ boldione
- ▶ calusterone
- ▶ clostebol
- ▶ danazol
- ▶ dehydrochloromethyltestosterone
- ▶ desoxymethyltestosterone
- ▶ drostanolone
- ▶ ethylestrenol
- ▶ fluoxymesterone
- ▶ formebolone
- ▶ furazabol
- ▶ gestrinone
- ▶ 4-hydroxytestosterone
- ▶ mestanolone
- ▶ mesterolone
- ▶ metenolone
- ▶ methandienone
- ▶ methandriol
- ▶ methasterone
- ▶ methyldienolone
- ▶ methyl-1-testosterone
- ▶ methylnoretestosterone
- ▶ methyltrienolone
- ▶ methyltestosterone
- ▶ mibolerone
- ▶ nandrolone
- ▶ 19-norandrostenedione
- ▶ norboletone
- ▶ norclostebol
- ▶ norethandrolone
- ▶ oxabolone
- ▶ oxandrolone
- ▶ oxymesterone
- ▶ oxymetholone
- ▶ prostanazol
- ▶ quinbolone
- ▶ stanozolol
- ▶ stenbolone
- ▶ 1-testosterone
- ▶ tetrahydrogestrinone
- ▶ trenbolone
- ▶ and other substances with a similar chemical structure or similar biological effect(s)

Endogenous

- ▶ androstenediol
- ▶ androstenedione
- ▶ dihydrotestosterone
- ▶ prasterone
- ▶ testosterone
- ▶ metabolites and isomers, including:
 - ▶ 5 α -androstane-3 α ,17 α -diol
 - ▶ 5 α -androstane-3 α ,17 β -diol
 - ▶ 5 α -androstane-3 β ,17 α -diol
 - ▶ 5 α -androstane-3 β ,17 β -diol
 - ▶ androst-4-ene-3 α ,17 α -diol
 - ▶ androst-4-ene-3 α ,17 β -diol
- ▶ androst-4-ene-3 β ,17 α -diol
- ▶ androst-5-ene-3 α ,17 α -diol
- ▶ androst-5-ene-3 α ,17 β -diol
- ▶ androst-5-ene-3 β ,17 α -diol
- ▶ 4-androstenediol
- ▶ 5-androstenedione
- ▶ epi-dihydrotestosterone
- ▶ 3 α -hydroxy-5 α -androstane-17-one
- ▶ 3 β -hydroxy-5 α -androstane-17-one
- ▶ 19-norandrosterone
- ▶ 19-noretiocholanolone

The WADA Prohibited List includes some chemical names, e.g., 18 α -homo-pregna-4,9,11-trien-17 β -ol-3-one for tetrahydrogestrinone, as well as criteria for reporting endogenous steroids of exogenous origin.

Most specialized labs that test athletes for steroids use gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS-MS). Chromatography separates the urine sample components, and mass spectrometry identifies them by determining molecular fragmentation patterns and by matching unknown data with reference data. This method identifies not only classes of compounds but also specific chemical structures. For example, a lab might report that a sample contained 3'-hydroxystanozolol, a stanozolol metabolite.

One limitation of these techniques is that they cannot distinguish natural steroids like testosterone from pharmaceutical testosterone. Fortunately, the ¹³C content of the two compounds differs, and gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) can detect this difference by first separating sample ingredients and then combusting them to convert all carbon atoms to CO₂.

The Cost of Steroid Testing

To conduct drug testing for the International Olympic Committee and other major sports organizations, labs must meet the requirements of ISO/IEC 17025 and be accredited by

WADA. Labs that handle a large number of urine samples can typically perform steroid testing for under \$100 per sample, assuming the positive rate is less than 1%. This price includes meeting the requirements of ISO/IEC 17025, maintaining accreditation by WADA, and performing steroid screening, A confirmation testing, and B confirmation testing. Legal costs such as documentation and testimony must also be factored in. This cost per sample, however, does not include the cost of urine collection and shipment to the lab.

Despite these expenses, programs that want to do this type of steroid testing can lower their cost per sample in several ways. For example, they might tolerate a longer turn-around time and allow the lab to report results within a month instead of a week after it receives samples, or shorten the target list of steroids and exclude detection of novel designer steroids.

The Legal Realities

While the logistics of steroid testing are straightforward, the legal repercussions of the results can be far more complex. After organizations that administer drug testing programs collect athletes' urine samples and ship them to the lab, the lab tests the samples and reports results to the organizations,

which apply sanctions as needed. Then, however, some athletes appeal their test results and the lab's participation in legal activities can range from preparing hefty legal documentation or witness statements to sending the lab director or multiple technicians to testify in court. While this may happen for only a few cases per year, the process is time consuming.

In addition, the need to maintain confidentiality, follow strict standard operating procedures, constantly document chain of custody, and address other legalities can feel burdensome to chemists. Yet among the thousands of samples tested at each WADA-accredited lab every year, any one could contain a banned drug and might lead to years of legal battles since a positive test result could crush an athlete's life and livelihood. The significance of such consequences therefore justifies the lab's need to make every effort to follow meticulous procedures.

Beyond the Test Results

Anabolic steroids can persist in the body for a period of time ranging from as short as a few days to as long as many months after the user stops taking them, depending on the type of steroid used—short-acting pill or long-acting injection—how much, and for how long. A urine test result shows what banned substances or metabolites are present in the urine at the time the sample was collected; however, the test result cannot show what brand or formulation of the drug was used; by what route, whether by mouth or injection; in what amount; or when—i.e., how long before the urine was collected.

Negative test results, on the other hand, indicate that the drug was never used or that any banned steroid was used sufficiently long ago that the body has completely eliminated it. Other possibilities for a negative result are that the level of an illegal steroid is below the limit of detection of the test or that the athlete took an illegal steroid for which the lab does not test. A urine test could also be negative if the sample is not real urine from the individual being tested or if the urine had been manipulated. However, labs that specialize

in steroid screening can detect tampering in samples devoid of natural steroids that would be missed by commercial dipsticks or other adulteration tests such as those performed by workplace drug testing labs.

In addition to addressing all possible causes of a negative result, labs must also address a common explanation given by athletes for a positive result: that they took a supplement, perhaps one having nothing to do with steroids, such as vitamins or proteins. However, the only steroids reported in peer-reviewed publications that have been found in contaminated supplements are testosterone, its precursors or prohormones—which the body metabolizes to the active hormone—and related 19-nor steroids. Indeed, scientific studies have shown that consuming the meat of a non-castrated boar or contaminated supplements can result in the presence of 19-norandrosterone, a prohibited steroid, in urine. This steroid can also be found in urine after administration of nandrolone, 19-norandrostenediol, or 19-norandrostenedione, all of which are prohibited steroids. In a few cases, athletes have had their supplements analyzed by independent labs, which have or have not found contaminants consistent with the adverse finding.

The Final Analysis

Last year's development in Major League Baseball brought the problem of steroid use in professional sports back into the public's—and Congress'—consciousness. Coupled with a growing concern about steroid use by college and high school athletes, steroid testing has become a highly visible area of laboratory testing.

To stop the use of steroids in athletics, however, laboratorians and sports organizations need to begin to think "outside the urine cup" and find alternatives to mandatory urine testing. One suggestion is that clean athletes might voluntarily undergo random drug testing and let their names appear on a published list. Such an open system could promise something tangible—health monitoring by physicians who track serum hormones and fitness markers. If one parameter

Anabolic Steroids Most Often Found in Sports Drug Tests

- ▶ 19-norandrosterone (found in approximately one third of all anabolic steroids cases world-wide)
- ▶ T/E>6 (found in a little less than one third of cases)
- ▶ Stanozolol (found in approximately one fifth of cases)

Source: WADA 2004 Adverse Analytical Findings Reported By Accredited Laboratories and WADA Statistics 2003.

moves out of line, the athlete would simply be asked to leave the program; he or she need not be accused of doping or suspended from competition. Only clean athletes would be likely to sign up for such a volunteer program. Rewarding clean athletes instead of punishing cheaters might turn the culture around and possibly curb the use of performance-enhancing drugs.

Until such alternative monitoring programs are enacted, however, testing the urine of athletes is the best way to catch those who use illegal steroids. **CLN**

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