Academe *Journal of Education & Psychology* *ISSN: 2249-040X*
Volume-16, (Special Issue: March 2026) *Peer Reviewed & Refereed Journal (IF: 6.25)*
PP: 433-441 *Journal Website:* www.academejournal.in

DOPING: DETECTION TECHNIQUES FOR BANNED SUBSTANCES IN SPORTS

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ABSTRACT

The use of illegal performance-enhancing drugs (PEDs) in sports, and other tactics by athletes to gain an unfair competitive advantage is becoming a global concern since it negatively impacts the athlete, participants, and the integrity of sports. Annually, a list of drugs and techniques that are prohibited from being used in sports is published by the World Anti-Doping Agency (WADA). The WADA list consists of different classes namely, non-approved substances, anabolic androgenic steroids (AAS), peptide hormones, growth factors, related substances and mimetics, beta-2 agonists, hormone and metabolic modulators, diuretics and masking agents, stimulants, narcotics, cannabinoids, glucocorticoids, and beta blockers. Moreover, athletes tend to use other cheating techniques such as blood doping, urine adulteration, and gene doping to increase their chances of winning.

Keywords: Drug Abuse, Doping, Performance-Enhancing Drugs, Dope Testing, Sports, Sports Medicines.

1. INTRODUCTION

Drug abuse in sport is habitually called as “doping”. The universal word „dope“ is being used both as a noun and as a verb. The word does not appear in this context before the twentieth century regardless of the practice of horse „nobbling“, which was known well before this time.

For example, the story of the famous trial of Daniel Dawson, publicly executed at Cambridge in 1812 for poisoning racehorses with arsenic. The abuse of drugs in an attempt to enhance performance in human sporting competitions is not new. For example, the Greek authors Phylostratos and Galen stated on the ethics of participants in the Olympics who would take any preparation to improve their performance. Roman gladiators were often intoxicated to make their fights more vigorous and gruesome as demanded by the spectators. The effect of drugs on performance is often exceptionally difficult to determine, and there is little conclusive available work for any species. The results that have been published are often contradictory as some of the workers advocate an increase in the competitor“s performance and others suggest no improvement. The assessment systems used to evaluate the effect of drugs may not sufficiently relate to the appropriate sporting performance, such as increase in muscle strength and sprint running.

Moreover, athletes may take far larger amounts of drugs than would be ethically acceptable in most human experiments. The toxic side effects of drugs are less difficult to ascertain, but the conclusions drawn from the available data are often contingent. However, there is sufficient evidence of the harmful effects when certain drugs are misused to validate their prohibition from sports competitions. In human sports, the main monitoring body is International Olympic Committee (IOC). Nevertheless, since 1999, doping concerns have been taken over by the World Anti-Doping Agency (WADA).

Doping with performance-enhancing drugs in both amateur and professional sports has become an increasingly growing problem. As the technology to detect drugs and illegal

substances has improved, an increased number of athletes are being caught out, as the limits of detection are more significant. Each sport has its own rules and regulations as to which substances are banned, but with the enhancement in drug detection, it has become harder for sportsmen and women to misuse drugs in athletics.

It is not just the use of prohibited substances that is considered doping. The World Anti-Doping Agency (WADA) has a strict code that defines the categories considered doping. The types of doping violation defined by WADA include:

a. The presence of a prohibited substance or its metabolites in a sample
b. Evading or refusing to give a sample
c. Missing tests and failing to provide information on missed tests
d. Tampering with samples or any part of the doping process
e. Possession of a prohibited substance or method
f. Trafficking of a banned substance or method
g. Administering or attempting to deliver prohibited substances or methods
h. Assisting and covering up any intentional complicity

2. FAMOUS SCANDALS

Over the years, there have been many well-publicized cases of doping in sport. Due to the nature of the sport, drug doping in athletics is quite common, but a lot of other sports also suffer at the hands of drugs cheats. Sports such as cycling, swimming, gymnastics, baseball weight-lifting and martial arts all have famous examples.

One of the biggest doping scandals to date is Russian doping scandal, which saw some athletes banned from the 2016 summer Olympics and only a select few being allowed to compete under a neutral flag at the 2018 winter Olympics.

To this date, there have been 47 medals stripped from Russian athletes that have competed at the Olympic level alone, as well as more than 200 athletes being caught out for doping, which is the highest of any country.

Outside of the Russian team, there have been many famous track and field athletes banned for doping. Olympic sprinting has a lot of cases of athletes testing positive for banned anabolic steroids. Cycling is another major sport that has seen instances of doping.

Doping in martial arts and boxing includes not only the steroids and growth hormones banned in other sports, but also recreational drug use is common due to the numbing effects of the drugs. Mixed martial arts are fraught with doping scandals, with many fighters having titles stripped and being given suspensions that prevent them from fighting.

3. COMMON DRUGS ABUSED

The types of drugs and substances that are abused include prescribed medications, recreational drugs, stimulants, steroids and hormones. Examples of the banned substances include:

- **Prescribed medication:** This area is commonly abused due to how easy it is to get false prescriptions.
- Recreational drugs such as alcohol, narcotics, marijuana and all cannabinoid derivatives

- Stimulants such as amphetamines and cocaine
- **Testosterone:** Although testosterone is an endogenous steroid, athletes are tested to make sure they do not have excess exogenous levels of testosterone in their bodies.
- **Anabolic Steroids:** One of the main groups of banned substances are anabolic steroids. They are synthetically manufactured drugs that mimic testosterone.
- **Human Growth Hormone (hGH):** hGH is a peptide hormone commonly used as it is hard to detect due to having such a short half-life. It is not a steroid but has an anabolic effect similar to steroids.

4. TEST METHODS USED TO DETECT PERFORMANCE- ENHANCING

Sports doping is not only contradictory to the concept of fair play, but it has a negative impact on the health of athletes as well as society in general. For these reasons, drug doping testing is conducted based on regulations imposed by the World Anti- doping Agency (WADA).

4.1 Urine testing

Urine testing is the most prevalent method for detecting prohibited substances in sports because it is non-invasive and allows for the detection of drugs and their metabolites at higher concentrations than in blood. The process is highly regulated by the World Anti-Doping Agency (WADA), ensuring that every sample is collected, handled, and analyzed under a strict chain of custody to maintain integrity.

Key Aspects of the Urine Testing Process

- **Observed Collection:** To prevent sample substitution or tampering, a Doping Control Officer (DCO) of the same gender must directly observe the urine leaving the athlete's body. Athletes are required to adjust their clothing from "knees to chest" to provide an unobstructed view.
- **Sample Integrity & Suitability:** A minimum of **90 ml** of urine is required for a complete sample. The DCO immediately checks the **specific gravity** (concentration) to ensure the sample is not too dilute for accurate laboratory analysis; if it fails this check, the athlete must provide additional samples.
- **A and B Sample Splitting:** The athlete personally divides their urine into two tamper-evident bottles labeled "A" and "B". The **A sample** is used for the initial analysis, while the **B sample** is securely stored as a backup to be tested only if the first result is positive and the athlete requests a second analysis for verification.
- **Laboratory Analysis (GC-MS and LC-MS):** Accredited laboratories use advanced techniques like **Gas Chromatography-Mass Spectrometry (GC-MS)** and **Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)** to separate and identify the molecular structures of prohibited substances. These methods are sensitive enough to detect minute traces of drugs long after they were ingested.

Examples of Substances Detected

Category	Specific Examples	Purpose of Use in Sport
Anabolic Agents	Testosterone, Stanozolol, Trenbolone	Increase muscle mass and strength.

Stimulants	Cocaine, Amphetamine, Modafinil	Enhance alertness and reduce fatigue.
Diuretics	Furosemide, Hydrochlorothiazide	Mask other drugs or rapidly lose weight.
Hormone Modulators	Anastrozole, Tamoxifen	Manage side effects of steroid use.
Narcotics	Fentanyl, Morphine, Oxycodone	Manage pain during intense training.

4.2 Blood testing

Blood testing complements urine analysis by detecting substances that are either too large to pass into urine or have extremely short lifespans in the body. While urine is great for finding drug breakdown products (metabolites), blood is essential for identifying large peptide hormones and direct evidence of blood manipulation, which can be nearly invisible in a urine screen.

Core Components of Blood Testing

- **Growth Factor Detection:** Blood is the primary matrix for detecting Human Growth Hormone (hGH) and Insulin-like Growth Factor-1 (IGF-1). These substances occur naturally, so labs must use specific "isoform tests" to distinguish between what the body makes and what an athlete injects.
- **The Athlete Biological Passport (ABP):** This is a longitudinal profile that tracks an athlete's **haematological markers** (like hemoglobin levels and reticulocyte counts) over months or years. Instead of looking for a specific drug, it looks for "biological fingerprints" of doping, such as sudden spikes that suggest EPO use or blood transfusions.
- **Detection of Erythropoiesis-Stimulating Agents (ESAs):** While some versions of **EPO** can be found in urine, blood testing provides a more accurate window for newer, continuous erythropoietin receptor activators (CERA) that stimulate red blood cell production.
- **Transfusion Detection:** Modern blood testing can identify "homologous" transfusions (blood from a donor) by looking for different **red blood cell surface antigens**. It can also detect "autologous" transfusions (the athlete's own stored blood) by measuring changes in cell age populations or the presence of plasticizers from storage bags.

Examples of Substances & Methods

Category	Specific Examples	Detection Goal
Peptide Hormones	hGH, GHRPs, IGF-1	Increasing muscle mass and recovery.

Blood Boosters	EPO, Darbepoetin alfa	Increasing oxygen-carrying capacity.
Oxygen Carriers	HBOCs, Perfluorochemicals	Artificially enhancing aerobic endurance.
Markers of Doping	Hemoglobin, Hematocrit	Identifying blood volume manipulation.
Newer Methods	Dried Blood Spots (DBS)	Testing for steroids/hormones via a finger prick.

4.3 Gas chromatography-mass Asapectrometry (GC-MS)

Gas Chromatography-Mass Spectrometry (GC-MS) is the "gold standard" for identifying small, volatile molecules in an athlete's sample. It works by combining two distinct technologies: the **Gas Chromatograph** separates a complex mixture into individual components, and the **Mass Spectrometer** identifies those components by breaking them into charged fragments. This "chemical fingerprinting" provides near-certain identification of prohibited substances even at extremely low concentrations.

How the GC-MS Process Works

- **Sample Volatilization:** The extracted sample is injected into the GC and heated until it turns into a gas. This gas is carried by an inert mobile phase (usually helium) through a long, thin column.
- **Separation (Retention Time):** As the gas travels through the column, different molecules move at different speeds based on their chemical properties. The time it takes for a specific substance to exit the column is its **retention time**, which serves as the first clue to its identity.
- **Ionization and Fragmentation:** As molecules exit the GC, they enter the Mass Spectrometer, where they are bombarded with electrons. This shatters the molecules into smaller, charged fragments called **ions**.
- **Mass Analysis:** The ions are accelerated through a magnetic or electric field that sorts them by their **mass-to-charge ratio (m/z)**. The resulting pattern of fragments (the mass spectrum) is unique to every chemical, much like a human fingerprint.

Examples of Use in Anti-Doping

Application	**Specific Examples**	**Why GC-MS?**
Anabolic Steroids	Nandrolone, Stanozolol	Detects specific metabolites that stay in the body for weeks.
Stimulants	Amphetamines, Ephedrine	Provides rapid, definitive identification in "in-competition" testing.
Narcotics	Morphine,	Distinguishes between closely related painkilling

	Codeine	compounds.
Isotope Analysis	Synthetic Testosterone	When coupled with an IRMS, it proves if testosterone is natural or injected.

4.4 Liquid chromatography- mass spectrometry (LC-MS)

Liquid Chromatography-Mass Spectrometry (LC-MS) is the preferred analytical technique for detecting substances that are "thermally unstable" (break down when heated) or have high molecular weights, making them unsuitable for the high temperatures required in Gas Chromatography. Instead of using heat to vaporize a sample, LC-MS uses a liquid solvent to carry the sample through a pressurized column, allowing for the detection of a much wider range of modern pharmaceuticals and large peptide hormones.

Core Components of the LC-MS Process

- **Liquid Phase Separation:** The sample is dissolved in a liquid (the mobile phase) and pumped at high pressure through a column packed with microscopic beads. Substances are separated based on how strongly they interact with these beads; some move quickly through the liquid, while others are "sticky" and take longer to emerge.
- **Soft Ionization (ESI):** Unlike GC-MS, which often shatters molecules using harsh electron beams, LC-MS typically uses **Electrospray Ionization (ESI)**. This "soft" technique applies a high voltage to the liquid as it exits the column, turning it into a fine mist and creating intact, charged ions. This is crucial for identifying large, fragile molecules like proteins.
- **Tandem Mass Spectrometry (MS/MS):** Most anti-doping labs use "Triple Quadrupole" systems. The first stage selects a specific "parent" ion, the second stage breaks that ion into pieces, and the third stage measures the resulting "daughter" ions. This double-layer of filtering eliminates chemical "noise," allowing scientists to find a single prohibited molecule hidden among millions of natural ones.
- **High Sensitivity for Trace Detection:** Because LC-MS does not require high heat, it can detect modern "designer" drugs and potent hormones that exist in the body at incredibly low concentrations (picograms per milliliter), which would be destroyed by other testing methods.

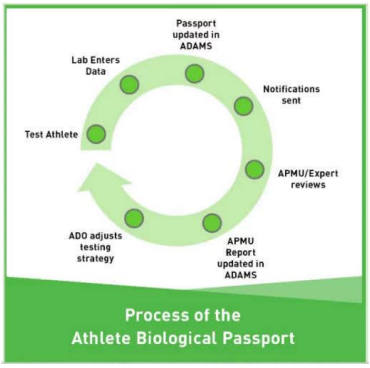
Examples of Substances Detected

A classic example is the detection of Corticosteroids (like Prednisolone), which are used to reduce inflammation but are prohibited in-competition; these molecules are too fragile for gas-based testing. Another major application is the screening for Diuretics (like Furosemide), used by athletes to flush their systems or meet weight classes, as these dissolve easily in the LC liquid phase. Finally, LC-MS is the primary tool for identifying Non-Proteolytic Growth Hormone Secretagogues, which are small molecules designed to trick the body into producing more natural growth hormone.

4.5 Athlete biological passport

The **Athlete Biological Passport (ABP)** is an indirect anti-doping method that monitors an athlete's biological variables over time to detect the *effects* of doping rather than searching for a specific prohibited substance. By establishing an individual's "normal" baseline, it identifies

suspicious fluctuations that could indicate the use of performance-enhancing drugs or methods like blood transfusions.



4.6 Isotope ratio mass spectrometry

Isotope Ratio Mass Spectrometry (IRMS) is the specialized "lie detector" of anti-doping. It is used primarily to determine if a substance found in an athlete's body—specifically testosterone or its precursors—is **endogenous** (naturally produced by the body) or **exogenous** (synthetic and administered from the outside). While standard GC-MS can tell *what* a substance is, IRMS tells you *where* it came from.

Examples of Use

A famous example of IRMS in action is its role in confirming "atypical" results from the Steroidal Module of the Athlete Biological Passport. If an athlete shows a naturally high Testosterone/Epitestosterone (T/E) ratio (which can happen genetically), IRMS is the definitive test used to clear them or catch them. Another example is the detection of Boldenone or DHEA; even if these substances are found at low levels, IRMS can prove they are not "natural fluctuations" but are chemically distinct from the athlete's own biochemistry.

4.7 Gene doping detection techniques

Gene doping is the non-therapeutic use of gene therapy to enhance athletic performance. Unlike traditional doping, which involves injecting a drug, gene doping involves injecting **genetic material** (DNA or RNA) or **modified cells** to trick the body into producing its own performance-enhancing substances, like extra EPO or muscle-growth factors.

5. ADVANCED DETECTION STRATEGIES

- **Direct Detection of Transgene DNA:** Scientists look for "transgenes"—synthetic DNA sequences used in gene therapy. Because synthetic DNA lacks the non-coding regions (introns) found in natural human DNA, labs can use **Quantitative PCR (qPCR)** or **Digital PCR (dPCR)** to find these "shorter" genetic sequences in an athlete's blood.
- **Viral Vector Testing:** Most gene doping requires a "delivery truck" (usually a modified virus like AAV) to get the DNA into the cells. Detection techniques can identify the presence of these specific viral proteins or the unique immune response (antibodies) an athlete's body produces after being "infected" by a gene-doping vector.
- **Transcriptomics and RNA Profiling:** Instead of looking for the gene itself, labs monitor **messenger RNA (mRNA)** levels. If an athlete has an unnaturally high "expression" of a specific gene (like the IGF-1 gene) without a medical explanation, it suggests the gene was artificially turned on.

- **Proteomic Signatures:** Gene doping causes the body to produce proteins that may be slightly different from natural ones. For example, "transgenic EPO" produced in muscle cells (after gene doping) has a different **glycosylation pattern** (sugar attachment) than natural EPO produced in the kidneys, which can be spotted using specialized electrophoresis.

Key Targets for Gene Doping

The most common targets for gene manipulation include Erythropoietin (EPO) for endurance, Myostatin inhibitors for massive muscle growth, and Vascular Endothelial Growth Factor (VEGF) to increase blood flow to muscles.

6. ROLE OF ACCREDITED LABORATORIES

WADA-accredited laboratories serve as the scientific backbone of the global anti-doping system. Their primary role is to conduct independent, high-precision analysis of samples to ensure that "clean" athletes are protected and those using prohibited substances are identified with legally defensible evidence.

Core Responsibilities of Accredited Labs

a. **Standardized Sample Analysis:** Laboratories must follow the **International Standard for Laboratories (ISL)** to ensure uniformity. Whether a sample is tested in Cologne, Tokyo, or Los Angeles, the analytical methods for screening and confirmation (like GC-MS and LC-MS) must meet the same rigorous sensitivity thresholds.

b. **Scientific Research and Method Development:** Labs do not just run tests; they are mandated to develop new techniques to stay ahead of "designer drugs." For example, they pioneered **Long-Term Metabolite (LTM)** testing, which extended the detection window for steroids from days to weeks.

c. **Expert Testimony and Results Management:** When a "Positive" (Adverse Analytical Finding) occurs, lab scientists provide the technical data and expert testimony required for legal hearings. They must prove that the **Chain of Custody** was never broken and that the chemical "fingerprint" is indisputable.

d. **Sample Storage and Re-analysis:** Labs are responsible for the long-term cryogenic storage of samples (often for up to **10 years**). As detection technology improves, these labs re-test old samples to catch athletes who used substances that were "undetectable" at the time of collection.

7. THE ACCREDITATION PROCESS

To maintain their status, laboratories undergo constant "blind" proficiency testing. WADA sends "spiked" samples containing unknown substances; if a lab fails to identify the drug or reports a "False Positive," their accreditation can be suspended or revoked immediately. This ensures that the laboratory’s integrity is beyond reproach.

Examples of Key Functions

An essential function is the management of the Athlete Biological Passport (ABP) data. Labs upload every blood and urine marker directly into the ADAMS database, allowing for the longitudinal tracking discussed earlier. Additionally, they perform Special Analysis requests, such as searching specifically for "masking agents" if a sample appears suspiciously dilute or chemically manipulated.

8. CONCLUSION

The detection of doping in sport is a vital component to creating a fair competitive environment for athletes. Educating athletes on the process of doping detection from start to finish may help them make better decisions when they are faced with doping, either intentionally or unintentionally. The doping detection process starts with the World Anti-Doping Agency and other Anti-Doping Organizations deciding what athletes should be tested and what they should be tested for, and ends with either a positive or negative doping test. Athletes' knowledge on the doping detection process often ends when their sample is collected, unless they are accused of doping and face consequences of suspension or even being banned from the sport.

However, athletes should be more knowledgeable on the testing procedures used once their samples are collected. Today, the most common tests used are gas chromatography-mass spectrometry, liquid-chromatography-mass spectrometry, and mass-spectrometry-mass spectrometry, among others. These techniques can be used to detect steroids, narcotics, stimulants, masking agents, contaminants of dietary supplements, and other substances on the WADA Prohibited List. Innocent athletes should be able to defend themselves if a test method gives a false-positive result, and understanding the accuracy of these test methods and how many different techniques there are should deter dishonest athletes from doping. Advancements are constantly being made to detect new substances and better detect substances that are commonly used. Informing athletes of the possible health risks and how certain drugs may even decrease their athletic performance should be deterrents as well. Educating the athletic community on the doping process from start to finish is the key to creating a doping free environment for competitive sports.

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