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CASE REPORT

A new series of hair test results involving anabolic steroids

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Summary In contrast with urine, hair analysis has a wide window of detection, ranging from weeks to months, depending on the length of the hair shaft, and provides information concerning the pattern of an individual's drug abuse. The Society of Hair Testing has published on 16th June 1999, a consensus opinion on the use of hair in doping situations but hair analysis is not yet recognized by the World Anti-Doping Agency (WADA), although this technology is accepted in most courts of Justice. While analysis of urine specimens cannot distinguish between chronic use or single exposure, hair analysis makes this distinction. This is very useful in case of identification in urine of restricted compounds. The major application may be in identifying false-negatives, since neither abstaining from a drug for a few days nor trying to "beat the test" by diluting urine, will alter the concentration in hair. Urine does not indicate the frequency of drug intake in subjects who might deliberately abstain for several days before screening. Doping during training and abstinence during the competition can therefore be detected, as athletes cannot evade the test. Hair testing should not be considered as an alternative to urinalysis but only as a complement in positive case to document the claim of the athlete, and it must be clear that in case of positive urine results, the negative hair result cannot exclude the administration of the detected drug and cannot overrule the positive urine result. In this compendium, 17 positive results from 73 requests for anabolic drugs testing in hair are presented. Boldenone (10 and 29 pg/mg), stanozolol (17–1992 pg/mg), methandienone (14–2800 pg/mg), methyltestosterone (12 and 22 pg/mg), clenbuterol (39 and 97 pg/mg), nandrolone (4–50 pg/mg), 19-norandrostenedione (4–13 pg/mg) and mesterolone (7 pg/mg) were identified during routine practice.

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Introduction

The use of stimulants (amphetamine, ephedrine, strychnine...) in sport to improve performances was reported in the early 1900s. The Medical Commission of the International Olympic Committee (IOC) established in 1967, the first list of prohibited substances and methods and adopted a Medical Code to protect the health of athletes and to ensure respect for the ethical concepts implicit in fair play, the Olympic spirit and medical practice. More recently, and after the Tour de France in 1998, the concerns in doping resulted in the formation of the World Anti-Doping Agency (WADA).

The current rules governing doping in sport have as their core that a doping violation is deemed to occur on finding in a body fluid a prohibited substance, a metabolite of a prohibited substance or a compound chemically or pharmacologically related to a prohibited substance. In most cases, urine is the specimen of choice, but recombinant human erythropoietin and related compounds or hormones can be detected in blood. To date, hair is not accepted in doping control, although France passed in 2001 a law allowing biologists to use this matrix to document doping (décret No. 2001-35 from 11th January 2001).

The major practical advantage of hair testing compared to urine or blood testing for drugs is that it has a larger surveillance window (weeks to months, depending on the length of the hair shaft, against 2–4 days for most xenobiotics). For practical purposes, the two tests complement each other. Urinalysis and blood analysis provide short-term information of an individual's drug use, whereas long-term histories are accessible through hair analysis. While analysis of urine and blood specimens cannot distinguish between chronic use or single exposure, hair analysis can offer the distinction.

Use of anabolic steroids was officially banned in the mid 1970s by sports authorities. The first control of anabolic steroids (particularly metandienone found in Dianabol®) was achieved in Montreal in 1976 during the Olympic games.

Anabolic steroids are detectable in urine only 2 to 4 days after exposure, except for the ester forms.

Anabolic steroids are prohibited, their presence in a urine sample lead to a positive result except for endogenous steroids (testosterone, epitestosterone, DHEA, nandrolone which have threshold of positivity). In hair, the parent compound is the target analyte that is the opposite of urine where the metabolites are of interest.

Only few papers dealing with the detection of anabolic drugs in human hair have been published, mostly focused on analytical procedures or focused to a single compound [1–9]. Therefore, it was our opinion to add some results to share with the scientific community. In 2014, we published the results of the first series of results [10]. It was our opinion, that 3 years later, some new data would be helpful for the scientists actively involved in this field of hair testing. Therefore, the present manuscript should be considered as an addendum to the previous article.

Specimens

Between September 2014 and September 2016, this office received 73 hair specimens with a view to assessing if there is any evidence of historical anabolic drugs abuse by the donor of the sample. The items were received sealed and the chain of custody was intact. The samples were logged onto the system and processed at the laboratory. At the request of the solicitor, the hair was examined, with the aid of scientific support staff for 19-norandrostenedione, mestrolone, methyltestosterone, DHEA, DHT, methenolone, THG, stanozolol, testosterone and esters, nandrolone and esters, methandienone, boldenone, clenbuterol and salbutamol over fixed period by the client. Self-reported drug consumption was not known. The use of cosmetics and hair sprays or lotions was sometimes indicated but cannot be considered as a general rule.

From 73 hair specimens, 21 were body hair (mainly chest hair, but also leg or under arm hair). The influence of gender was not considered to be of importance and was not always provided.

Analyses

All analyses were achieved under ISO17025 accreditation, using a previously published method [3], recently modified according to our previous paper [10].

In most cases, the reason for steroids testing was unknown. Less than 10% were based upon solicitors' requests. Three cases involved athletes having an adverse analytical finding in urine.

Briefly, 50 mg of finely cut hair was weighted. Internal standards were added together with methanol for a two-hour ultrasonic bath. Then, in one side, the organic phase was evaporated and diluted with phosphate buffer pH 6.8 (part A), and in another side, the remaining hair was incubated with 1 M NaOH to be melted (part B). Liquid-liquid extractions were operated on parts A and B with ethyl acetate. The aqueous A phase was isolated and 1 M NaOH added before extraction with ethyl acetate. The three organic phases were combined and purified on NH₂ cartridge. The final phase was evaporated and diluted with ethyl acetate to be divided into two parts: for injection onto GC-MS/MS apparatus (TSQ 8000) after evaporation and TMS derivatization, and for injection onto LC-MS/MS apparatus (TSQ Quantum) after evaporation and dilution with methanol.

Results and discussion

Extensive chromatographic procedures (two purification steps by solid-phase and liquid-liquid extractions, MS-MS detection) were analytical prerequisites for successful identification of anabolic steroids in hair due to the low target concentrations.

Testosterone and DHEA were always detected at physiological concentrations (lower than 10 and 15 pg/mg, respectively), confirming our previous studies [11,12]. Normal concentrations of testosterone in hair are generally

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