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HISTORICAL DEVELOPMENT OF ANALYTICAL METHODS FOR ANTI-DOPING CONTROL

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SUMMARY

Although the fight against the use of doping in sport has been going on for almost 90 years, its effects have become tangible in the last 45 years only, thanks to the use of valid and sensitive analytical methods. Historically, extensive international scientific cooperation and technological progress have laid down the basis for the development of high quality doping control laboratories worldwide. New biotechnology products are constantly being discovered and are made available on the doping market, so that anti-doping approaches must be raised to a higher level, and analytical methods must be constantly improved and refined, since it has bacome obvious that to some extent they lag behind new sophisticated doping agents. However, all the methods must first be scientifically proven and tested in order to be adequately used against doping in sport. If the technology and systematic use of the latest scientific anti-doping knowledge continue to develop and advance, it will greatly contribute to the development of analytical methods.

Key words: doping, anti-doping, analytical methods.

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INTRODUCTION

As early as 1928, during the Olympic Games in Amsterdam, the International Amateur Athletic Federation forbade the use of stimulative substances, becoming therefore the first of the sports federations which stood up

against doping in sport. Not long after that, the International Olympic Committee became aware of the need for constant surveillance of the issue of doping at the Olympic Games. This surveillance practice began after the session of the International Olympic Committee (IOC) in Warsaw and Cairo in 1937 and 1938 [1]. In 1938, in one of the IOC reports, the following statement was written: „The practice of doping is entirely under the IOC surveillance, and any person accepting or offering to supply dope should not be allowed to enter amateur competitions or the Olympic Games" [1]. After the IOC officially banned doping, almost all IOC member states began working to ban and suppress the use of doping at their national levels, attempting to regulate via appropriate laws the fight against doping in sports. However, the principal problem making difficult the activity of the IOC against doping in sport has been the absence of testing procedures to detect doping. The Medical Commission of the IOC was established in 1967 [1], with the purpose to monitor the reports of abuse of stimulants and other agents capable of improving the competitive output of athletes [2]. The Commission, headed by the Belgian Prince Alexandre de Merode, implemented the first doping control procedure at the Olympic Games in Grenoble and Mexico City in 1968. At these games, the athletes were tested for the presence of stimulative substances (alcohol, amphetamin, heroin, and cocain) in their body. A Swede, Hans Gunnar Liljenwall, competing in athletic pentathlon, was found to be positive for the presence of alcohol and he was stripped of the bronze medal he had won in the discipline. Doping control and analysis at these games can be considered a kind of a pilot-project; a systematic doping control and analysis in all sports was implemented at the Olympic Games in Munich in 1972[3].

DEVELOPMENT OF ANALYTICAL METHODS FOR ANTI-DOPING CONTROL

Despite the initial attempts, utilizing probably the chemical tests in test tubes, gas chromatography was the only one sufficiently reliable method at the Olympic Games in Munich in 1972, which, if positive, could possibly lead to the

[1] Dirix, A., & Sturbois, X. (1998). The First Thirty Years of the International Olympic Committee Medical Commission, 1967-1997. Laussane: IOC.

[2] Dirix, A. (1966). The doping problem at the Tokyo and Mexico City Olympic Games. The Journal of Sports Medicine and Physical Fitness, 6(3), 183-6. Donike, M., Clasing, D., & Klümper, A. (1974). Dopingkontrollen bei den Spielen der XX. Olympiade München 1972–Teil 2. Leistungssport, 4(3), 192-199.

[3] Donike, M., Clasing, D., & Klümper, A. (1974). Dopingkontrollen bei den Spielen der XX. Olympiade München 1972–Teil 2. Leistungssport, 4(3), 192-199.

punishment of the offender. Mass spectrometry as one of the analytical methods was already being used at the time, but it was not included among the anti-doping analyses. Professor Manfred Donike, as a head of the Institute of Biochemistry, University of Cologne, Germany, advocated the introduction of mass spectrometry among the anti-doping analyses from 1977 until his death in 1995 [4]. The establishment of a laboratory in Cologne in 1983 created a basis of a kind for the exchange of new ideas and results at a scientific forum between the IOC and the accredited laboratories of the World Anti-Doping Agency (WADA). His active involvement and engagement in numerous international federations and the IOC paved the way for the development of a quality doping control in the laboratories worldwide. As the result of extensive international scientific cooperation and technological progress, the combination of gas chromatography and mass spectrometry has become the leading analytical method used in laboratories to detect and quantify most of the doping substances, representing at the same time the gold standard in the identification of small molecules. The method is a combination of high separation power of chromatography and high information capacity and high sensitivity of mass spectrometry [5-6].

At the beginning of the 1970s, androgenic-anabolic steroids could not be detected using gas chromatography and mass spectrometry since these methods were not convenient for large-scale testing. This led to the development of immunoassay screening tests. Immunoassay screening tests started in 1974 at the European Athletics Championships, and was used at the Olympic Games in Montreal, Canada, 1976 [7]. In the same year, androgenic-anabolic steroids were added to the List of Prohibited Substances of the IOC. Although the evidence of abuse of androgenic-anabolic steroids in many sports had been present long before 1975, this group of substances was added to the list when the analytical methods to detect them were shown to be sufficiently reliable and sensitive. However, immunoassay methods could detect many, although not all synthetic

[4] Hemmersbach, P. (2008). History of mass spectrometry at the Olympic Games. *Journal of Mass Spectrometry*, *43*(7), 839-853.

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steroids, popular at the time [8]. Moreover, the combination of gas chromatography and mass spectrometry was still not ready in practice to confirm the results obtained by using immunoassay methods. The methodology of anti-doping control was thus such that the samples obtained from athletes were analyzed in the laboratories using immunoassays for screening purposes, but the obtained positive results were considered as preliminary. The final confirmation was performed using the combination of gas chromatography and mass spectrometry [9]. Although the methods of gas chromatography and mass spectrometry have been considerably modified since then [9], the principles of work have remained the same [10].

In the period between the Olympic Games in Moscow in 1980 and the Games in Los Angeles in 1984, the extensive studies of the profile of degradation steroids in the urine were performed. It was established that human urine contained a T isomer without any known biological function, epitestosteron. Soon after that, it was agreed that for the detection of androgenic-anabolic steroid abuse the ratio of testosteron and epitestosteron should be used, determined using the method gas chromatography-mass spectrometry. The first analysis performed using this methodology at the Olympic Games in Los Angeles in 1984, yielded 16 positive results [11]. Since then, urinary steroid profile analysis has become an essential tool in the fight against doping in sport[12]. The WADA set the cut-off value of the ratio of testosteron/epitestosteron higher than 4:1 as the one breaching the World Anti-Doping Code in sports. One of the problems in the assessment of ratio of testosteron/epitestosteron (T/E) is that some individuals, even without any intake of androgenic-anabolic steroids, can have elevated T/E ratio, while another related problem is that T/E never exceeds 4 in some of the

[8] Dugal, R., Dupuis, C., & Bertrand, M. J. (1977). Radioimmunoassay of anabolic steroids: an evaluation of three antisera for the detection of anabolic steroids in biological fluids. *British Journal of Sports Medicine*, *11*(4), 162-169.

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abusers of androgenic-anabolic steroids, which is probably due to some genetic factors [13].

IMPROVEMENT OF ANALYTICAL METHODS FOR ANTI-DOPING CONTROL

In the preparations for the Olympic Games in 1988, a laboratory in Seoul actively developed the method of gas chromatography-mass spectrometry for stimulants and narcotics [14,15]. Moreover, the abuse of beta-blockers was monitored for the first time using the improved method of gas chromatography-mass spectrometry in the selected ion-monitoring regime, as well as diuretic abuse, using high-performance liquid chromatography. During the 1990s, the methods of isotope-ratio mass spectrometry [16] and high-resolution mass spectrometry [17] were introduced among the anti-doping analyses, which made possible the detection of low concentration long-term metabolites of androgenic-anabolic steroids in the urine. The combination of liquid chromatography and mass spectrometry offers numerous advantages compared to gas chromatography-mass spectrometry, becoming the leading analytical method although poorly adjusted for the detection of some of the androgenic-anabolic steroids. A large number of banned substances in sports have been identified using gas chromatography-mass spectrometry or liquid chromatography-mass spectrometry, with the exception of glycopeptide or peptide hormones, such as erythropoietin and growth hormone.

[13] Jakobsson-Schulze, J., Lundmark, J., Garle, M., Skilving, I., Ekström, L., & Rane, A. (2008). Doping test results dependent on genotype of UGT2B17, the major enzyme for testosterone glucuronidation. *The Journal of Clinical Endocrinology Metabolism*, *93*(7), 2500-2506.

[14] Lho, D. S., Hong, J. K., Paek, H. K., Lee, J. A., & Park, J. (1990). Determination of phenolalkylamines, narcotic analgesics, and beta-blockers by gas chromatography/mass spectrometry. *Journal of Analytical Toxicology*, *14*(2), 77-83.

[15] Lho, D. S., Shin, H. S., Kang, B. K., & Park, J. (1990). Systematic analysis of stimulants and narcotic analgesics by gas chromatography with nitrogen specific detection and mass spectrometry. *Journal of Analytical Toxicology*, *14*(2), 73-76.

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[17] Schänzer, W., Geyer, H., Fußhöller, G., Halatcheva, N., Kohler, M., Parr, M. K., Guddat, S.,Thomas, A., & Thevis, M. (2006). Mass spectrometric identification and characterization of a new long-term metabolite of metandienone in human urine. *Rapid Communications in Mass Spectrometry*, *20*(15), 2252-2258.

The first attempts in the fight against erythropoietin abuse consisted in the introduction of indirect blood tests as an anti-doping procedure. The simplest indirect test consisted of hemoglobin and hematocrit measurements. The test was indirect, since it did not detect the presence of recombinant human erythropoietin, but only the traces of its use. A significant step forward was made in 2000, when *Lasne* and *de Ceaurriz* described a method based on isoelectric focusing [18]. At the winter Olympic Games in Salt Lake City in 2002, using the method based on isoelectric focusing, darbepoietin alpha (a synthetic form of erythropoietin) was successfully identified [19]. Nevertheless, the most effective way to eliminate blood doping is probably the introduction of biological passports that would enable the monitoring of variations of indirect markers (as required by the International Cycling Union since 2008 for the top level competitive cyclists).

A new analytical anti-doping method, used for the first time in Athens in 2004, is based on the detection of abuse of synthetic recombinant human growth hormone in samples [20]. The so called isoform method is based on the detection of growth hormone isoforms. The testing for a disturbed ratio of two natural growth hormone isoforms in the plasma (of 20 kDa and 22 kDa) using the method of liquid chromatography-tandem mass spectrometry is considered a significant step in the detection of growth hormone abuse[21].

CONCLUSION

Aided by the more reliable methods to detect doping and with permanent education of all individuals involved in sports about the general and far-reaching consequences of doping in sports, it is possible to turn the evolution of sport in a good direction. Regretfully, at this time, the proponents and users of doping are ahead of those against it, in spite of all anti-doping efforts and programs and the advancement of detection technologies in the last decade. The fight against

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[20] Wu, Z., Bidlingmaier, M., Dall, R., & Strasburger, C. J. (1999). Detection of doping with human growth hormone. *The Lancet*, *353*(9156), 895.

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substance abuse in sports has not yet yielded the expected results, but the validity of its purpose keeps it alive and current. Due to the constant discovery of new biotechnological products, commercially available on the doping market, the whole anti-doping approach has to be raised to a higher level, and analytical methods have to be constantly improved and refined to be able to cope with the new, more and more sophisticated doping agents. However, all the methods to be used adequately for the purpose, first have to be scientifically validated and confirmed. If the development of technology and systematic use of the current scientific knowledge continue, this would certainly contribute to the refinement of analytical methods. New, meticulously devised and conducted studies, with close observation of the relevant ethical principles, are the basis for the positive aspects of development of sports and sport competition in general.

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ИСТОРИЈСКИ РАЗВОЈ АНАЛИТИЧКИХ МЕТОДА АНТИДОПИНГ КОНТРОЛЕ

САЖЕТАК

Иако борба против коришћења допинга у спорту правно траје већ скоро 90 година, суштински се она реализује тек задњих 45 година, захваљујући

примени валидних и сензитивних аналитичких метода. Историјски гледано, као резултат опсежне међународне научне сарадње и технолошког напретка, постављена је основа за развој квалитетне допинг контроле у лабораторијама широм света. Због нових биотехнолошких производа стално присутним на допинг тржишту, целокупни антидопинг приступ се мора подићи на виши ниво, а аналитичке методе морају стално напредовати, јер је очигледно да у одређеној мери касне за новим софистицираним допинг средствима. Међутим, све методе морају бити прво научно доказане и проверене да би се могле адекватно користити против допинга у спорту. Уколико се развој технологије и систематско коришћење најновијих научних сазнања у сврху антидопинга наставе, то ће у великој мери допринети усавршавању аналитичких метода.

Кључне речи: допинг, антидопинг, аналитичке методе.

ИСТОРИЯ РАЗВИТИЯ АНАЛИТИЧЕСКИХ МЕТОДОВ АНТИДОПИГОВОГО КОНТРОЛЯ

АННОТАЦИЯ

Хотя борьба против применения допинга в спорте длится почти 90 лет, по сути, она происходит только в последние 45 лет благодаря использованию эффективных и чувствительных аналитических методов. Исторически широкое международное научное сотрудничество и технологический прогресс создают основу для развития контроля за качеством допинг-лабораторий по всему миру. Новые биотехнологические продукты постоянно появляются на рынке допинга, поэтому допинг-контроль и аналитические методы должны быть подняты на более высокий уровень, нужно постоянно двигаться вперед, поскольку совершенно очевидно, что допинг-контроль может опаздывать при появлении более изощренных допинг-препаратов. Однако, все методы должны быть научно доказаны и протестированы для того, чтобы их в дальнейшем адекватно можно было применять против допинга в спорте. Если развитие технологий и систематическое использование новейших научных антидопинговых знаний непрерывно будет идти вперед, то оно внесет значительный вклад в развитие аналитических методов антидопингового контроля.

Ключевые слова: допинг, анти-допинг, аналитические методы.

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