

Doping prevention – methods & analysis, trend of development



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[agenda]

- **Liquid chromatography – tandem mass spectrometry (LC-MS/MS)**
- **SARM**
- **Gene doping**

[LC-MS/MS detection]

- 1. AAS (e.g. Methyltrenbolone; “11 cases in 2008”)
- 2. SARM
- 3. PPAR δ -agonists (e.g. GW1516) = gene doping
- 4. Peptide hormones (e.g. Gonadorelin = LHRH)
- in blood and in urine
- advantages: high resolution & precision

[SARM selective androgen receptor modulators]

- 1. „positive-tested“ athlete
- in-competition
- Andarine (S4™)/GTx
- test developed from Cologne-Lab (Prof. M. Thevis)
- first detection in 10/09 in a sample with contamination



Bobby-Gaye Wilkins (r.): „AAF“ in March 2010 (Indoor-WWC of Athletics in Doha)

[SARM possible medical indications]

- **Tumor cachexia**
- **Sarcopenia**
- **unvoluntary loss of muscle mass (immobilization)**
- **Anti-conception in male**

[SARM substances & producers]

- **GTx (Memphis/Tennessee)**
- **Merck Pharmaceuticals/USA**
- **Andarine (S4™)**
- **Ostarine**
- **Non-industrial, possibly criminal production without low quality standards => cross-contamination, incalculable side-effects**

[SARM Andarine & Ostarine]

- **“SARM = side-effect free anabolic steroid” on web-site of GTX**
- **obviously severe side effects on vision with Andarine**
- **GTX & Merck stopped further development**
- **Ostarine (phase IIb closed)**
- **Nevertheless both on the black market via internet available**

[Gene doping classification of analysis]

- 1. use of genetic modified substances (“gene-therapy”): e.g. Repoxygen
- 2. modification of gene activity (Expression): PPAR δ -agonists like GW1516 + AICAR
- In future 3. (actually under 1): use of transfer-sequences (various RNA, e.g. siRNA = small interfering RNA)

[Gene doping 1 new test]

- *Beiter T, Zimmermann M, Fragasso A, Hudemann J, Niess AM, Bitzer M, Lauer UM, Simon P. Direct and long-term detection of gene doping in conventional blood samples. Gene Ther. 2010 Sep 2, online, doi:10.1038/gt.2010.122*
- *Beiter T, Zimmermann M, Fragasso A, Armeanu S, Lauer UM, Bitzer M, Su H, Young WL, Niess AM, Simon P. Establishing a novel single-copy primer-internal intron-spanning PCR (spiPCR) procedure for the direct detection of gene doping. Exerc Immunol Rev. 2008;14:73-8*

[Gene doping 1 new test]

- **basic idea behind: detection of transgene DNA (tDNA) via lack of Introns (non-coding sequences) => class 1 in actual classification**
- **clear „Yes or No-answers“**
- **maybe available until 2012**
- **Support by WADA**

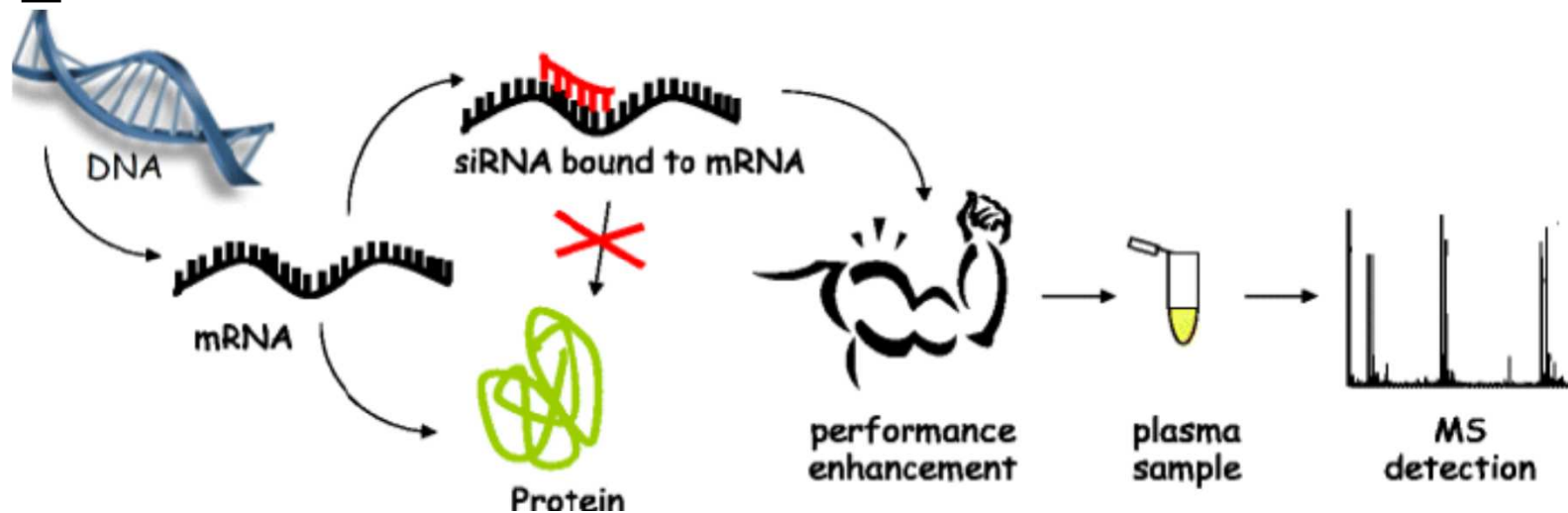
[Gene doping 2 test for GW1516]

- ***Thevis M, Beuck S, Thomas A, Kortner B, Kohler M, Rodchenkov G, Schänzer W.: Doping control analysis of emerging drugs in human plasma - identification of GW501516, S-107, JTV-519, and S-40503. Rapid Commun Mass Spectrom, 23(8) (2009)1139-46.***
- ***Thevis M, Möller I, Thomas A, Beuck S, Rodchenkov G, Bornatsch W, Geyer H, Schänzer W. Characterization of two major urinary metabolites of the PPARdelta-agonist GW1516 and implementation of the drug in routine doping controls Anal Bioanal Chem. 2010 Apr;396(7):2479-9.***

[Gene doping 2 test for AICAR]

- **Validation tests are necessary**
- **AICAR is produced in human beings too**
- *Thomas A, Beuck S, Eickhoff JC, Guddat S, Krug O, Kamber M, Schänzer W, Thevis M. Quantification of urinary AICAR concentrations as a matter of doping controls Anal Bioanal Chem 2010:2899-2908.*

Gene doping 3 siRNA-test in future



siRNA, which blocks the translation of genes, could be used by athletes for illicit performance enhancement by e.g. down-regulating the myostatin gene for enhanced muscle growth.

*Kohler M, Thomas A, Walpurgis K, Schänzer W, Thevis M.
Mass spectrometric detection of siRNA in plasma samples for doping control purposes.*

Anal Bioanal Chem, 2010 Jul 25, online doi 10.1007/S00216-010-4013-0

D. Doerr 2010

[Future of analysis in general]

- **actually almost only direct methods in use**
- **indirect methods to detect manipulated parameters as non-natural**
- **to proof a violation of anti-doping rules**
- **court-resistant?**