Anti-doping analyses in WADA-accredited laboratory

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La structure du CURML
LAD organogram

- 25 staff members
- 4 technicians trainees
Doping control

- **World Anti-Doping Agency (WADA, 1.1.2004)**
  - Harmonized and coordinated doping testing
  - Enhanced control and reporting

- **National anti-doping organizations (NADOs)**

- **National and international sport federations**

- **Event organizers**
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<th>Laboratory network – accredited laboratories</th>
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Laboratory network – accredited laboratories
Testing of athlete

- Organised and performed by **testing authorities** (TA)

- **Out-of-competition** and **in-competition** testing
  - Mainly urine samples, but recently also blood (serum, WB)

- **Minimum required urine sample volume 90 ml**
  - Divided in A- and B-containers, identification by sample code
  - Test form linking the athlete to the sample

- **Sealed** containers and **controlled** transport to laboratory
  - Chain-of-custody form
  - Temperature control and time constrains for blood samples
Sample containers

http://berlinger.ch
World Antidoping Agency documents

General documents:
- World Anti-Doping Code
- Guidelines
- Best practices

Specific documents:
- Prohibited List
- Laboratories
- Testing and Investigations
- Protection of Privacy and Personal Information
- Therapeutic Use Exemptions

Technical documents:
Prohibited List

1 Performance enhancer
2 Real or potential health risk
3 Use against ethic in sport

In-/out-of-competition
- S0 Non approved substances
- S1 Anabolic androgenic steroids
- S2 Hormones
- S3 β2-agonists
- S4 Hormone antagonist
- S5 Diuretics and other masking agents

In-competition
- S6 Stimulants
- S7 Narcotics
- S8 Cannabinoids
- S9 Glucocorticosteroids
- P2 β-blockers

Prohibited methods
- M1 Enhancement of oxygen transport
- M2 Chemical and physical manipulation
- M3 Gene doping

> 300 doping agents
Metabolism

Methandienone
Doping Control Analysis

- **Initial testing procedures (ITP, screening)**
  - Rapid, general and sensitive method for a group of compounds
  - Simultaneous analysis of several samples in one batch

- **Confirmation procedures**
  - Identification of a single compound
  - Typically more specific and laborious than screening

- **B-analysis**
  - Performed only upon request from B-container
  - Analytical methods identical to A-sample confirmation
Characteristics of doping analysis

• **Anonymous samples**
  - Unique code for each A- and B-sample pair
  - Collection date, test event, sport, and gender
  - Specific transportation requirements (e.g. temperature logger)

• **Analytical batches**
  - Several samples processed in one batch
  - Limited sample volume
  - List of prohibited substances
  - Mainly qualitative analysis

• **Traceable chain-of-custody control**
  - For containers (DCO – laboratory – disposal)
  - For each sample aliquot (primary sample – process – disposal)
Analytical procedures – A- and B-samples

A : PAAF
A : negative
→ Report

B sample confirmation
B : AAF Sanction
B : negative

B sample analysis Consent, Sanction

A : AAF
A : negative
→ Report

AAF ≠ ADRV
Analytical procedures – multiple methods

Distribution urine (exogenous)

- Hydrolysis
  - CF+AN
    - 5mL urine + hydrolysis + LLE
    - Split evaporation
    - Derivatization GC-MS/MS
    - LC-MS/MS C18, 1.7μm, 100x2.1mm
  - DS
    - 100μL urine + 900μl water + IS
  - PE
    - 1mL urine + SPE WCX
  - NW
    - 1mL urine + SPE HLB ou MCX
- No hydrolysis
  - NC
Analytical process

1. **Sample preparation**
   - Solid-phase extraction (SPE)
   - Liquid-liquid extraction (LLE)
   - Special techniques (e.g. IAC)

2. **Chromatographic separation**
   - Gas chromatography (GC)
   - Liquid chromatography (LC)

3. **Detection**
   - Mass spectrometry (MS)
Analytical instrumentation

- GC/MS (single quadrupole)
- GC-MS/MS (triple quadrupole)
- LC-MS/MS (triple quadrupole, q-TOF)
- LC-MS (HRMS; QExactive, TOF)
- GC-C-IRMS
- Immunoanalyzers
- Electrophoretic methods
Technical Documents

Decision Limits for the Confirmatory Quantification of Threshold Substances

→ Quantitative performance

Minimum Required Performance Levels for Detection and Identification of Non-Threshold substances

→ Sensitivity
Laboratory data management

- **Laboratory Information Management System (LIMS)**
  - Unique internal code given to each sample
  - Process flow management by LIMS (work lists, results, delays)
  - Interfacing with analytical instrument for data transfer
  - Support in non-analytical laboratory activities

- **Data clearinghouse of WADA (ADAMS)**
  - Combines all the operators in one platform
    - Athlete whereabouts information
    - Doping control management (ADO)
    - TUE management (ADO)
    - Information clearing house
  - All the laboratory data are uploaded into ADAMS
    - Transparency in reporting
    - Athlete biological passport information
    - Statistics
Reporting and result management

• **Analytical results**
  - Submitted via ADAMS database
  - Negative result → 1) testing authority, 2) WADA
  - Adverse analytical finding (AAF) → TA, WADA, International Federation

• **Result management authority**
  - Sample code connects the result with the athlete

• **Follow-up**
  - Full laboratory documentation (for AAF)
  - B-analysis, unless the athlete waives her/his right
  - Disposal or long-term storage
Long-term storage of samples

• **WADA-regulations and standards**
  - Storage strategy made by testing authorities
  - Practical storing made by laboratories (transfers, conditions, chain of custody)
  - Potential retesting up to 10 years → deterrence effect

• Factors related to **prohibited substances**
  - Emerging compounds in official/illegal market
  - Research information on metabolism of traditional substances

• Factors related to **analysis techniques**
  - Completely new instrument technologies
  - Improved sensitivity of already applied systems
Research activities

• Improvement of biological passports
  ➢ New markers and confounding factors for sensitivity and specificity

• Analytical advances in instrument techniques
  ➢ GC/MS\textsuperscript{n} and LC/MS\textsuperscript{n}

• Forensic analyses
  ➢ DNA-analysis, product analyses for case management

• Gene doping
  ➢ On hold for the decisions of WADA
Thank you!