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What analytical tests
to order and when



Kuuranne T.

WADA ABP Symposium

November 5th-7th, 2018

Rome

Outline

- Analytical context – What and how?

- Prohibited list \neq target compounds
- Methods and instruments of choice



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- Routine test menus – Why not just to analyse everything at once?

- Complementary information from various sample matrices
- Capabilities and limitations of mass spectrometry in analytical routine



- Extended test menus – What to add, when and how?

- Evaluation of an altered biomarker vs. identification of an exogenous substance
- Systematic review of available data for correct analytical test

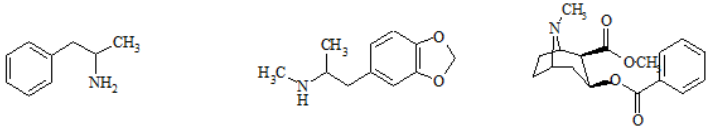


chemistryworld.com

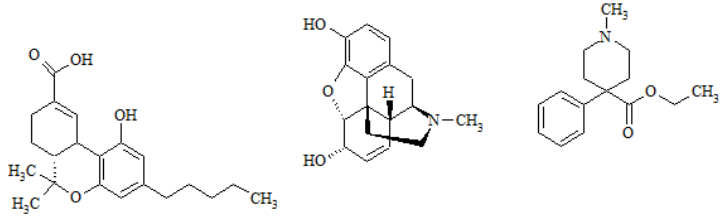


Prohibited substances - IC only

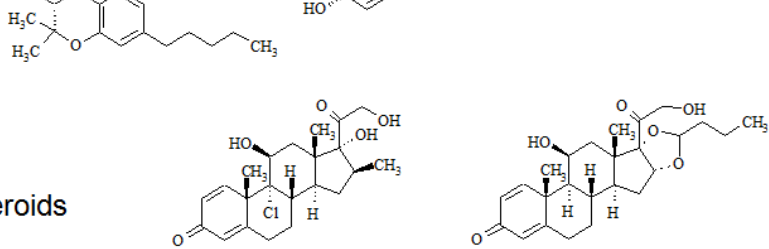
S6) Stimulants



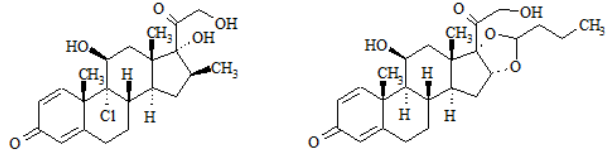
S7) Narcotics



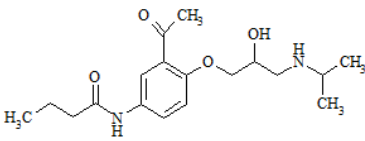
S8) Cannabinoids



S9) Glucocorticosteroids

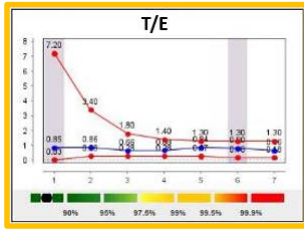


P2) Beta-blockers



Prohibited substances - IC and OOC

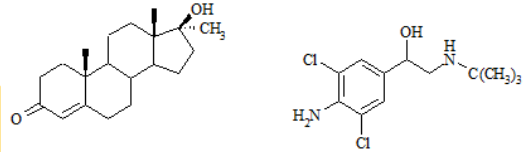
Endogenous AAS



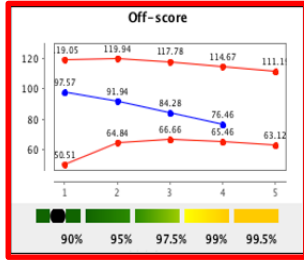
S0) Non-approved substances

S1) Anabolic agents
1 Anabolic androgenic steroids

2 Other anabolic agents



Agents affecting erythropoiesis

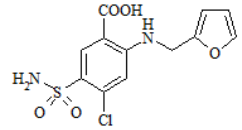
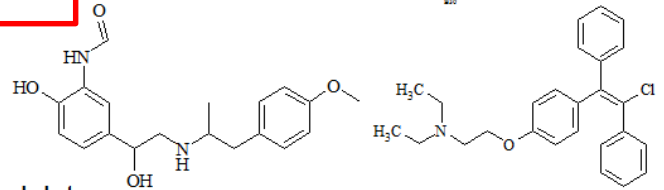
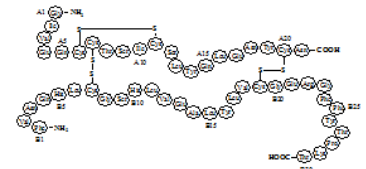


S2) Hormones and related substances

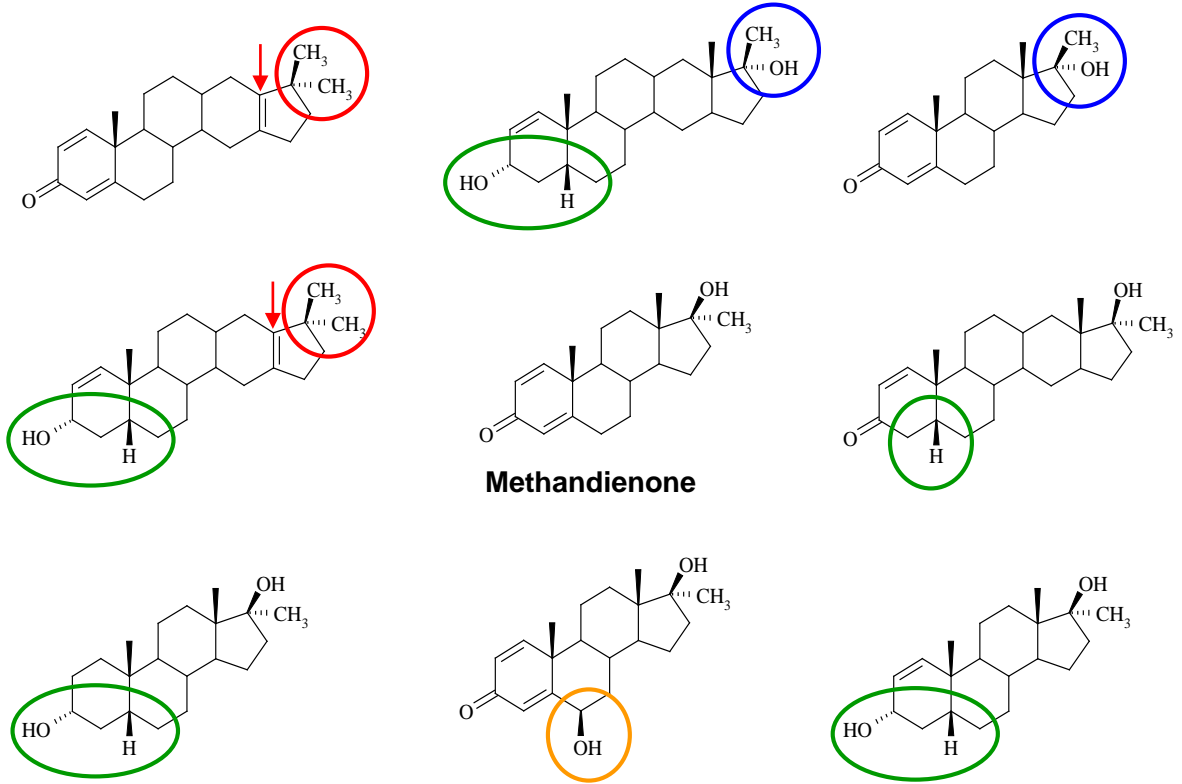
S3) β_2 -agonists

S4) Hormone antagonists and modulators

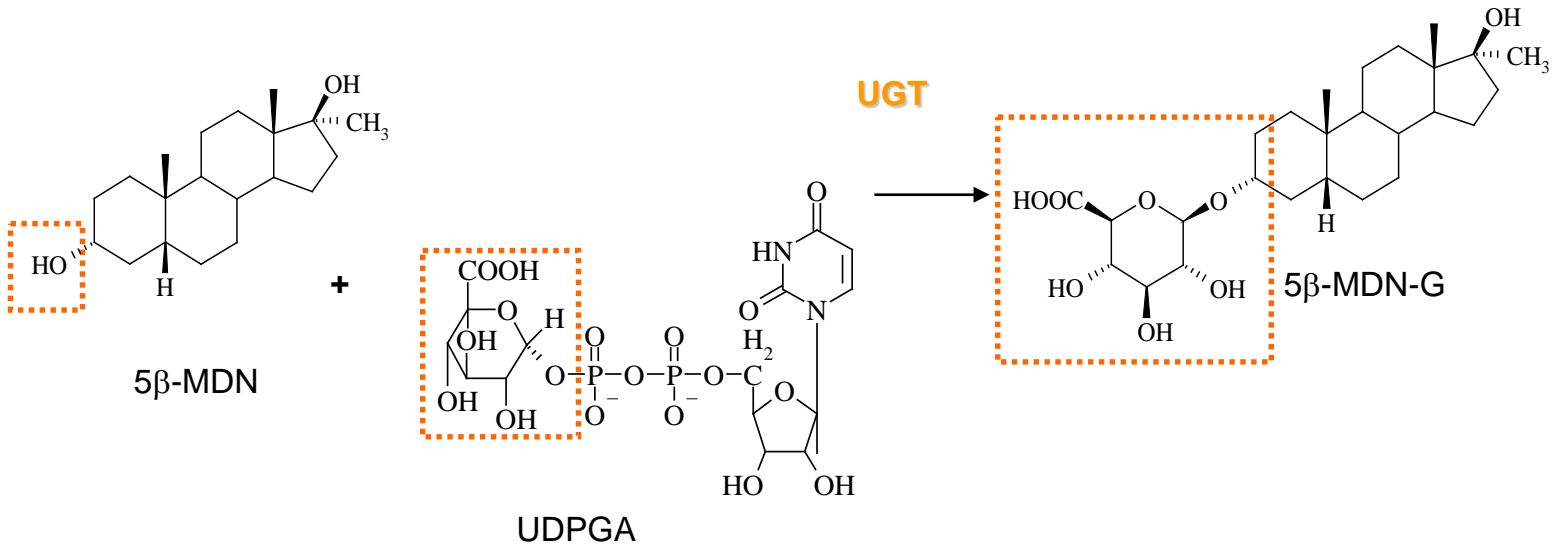
S5) Diuretics and other masking agents



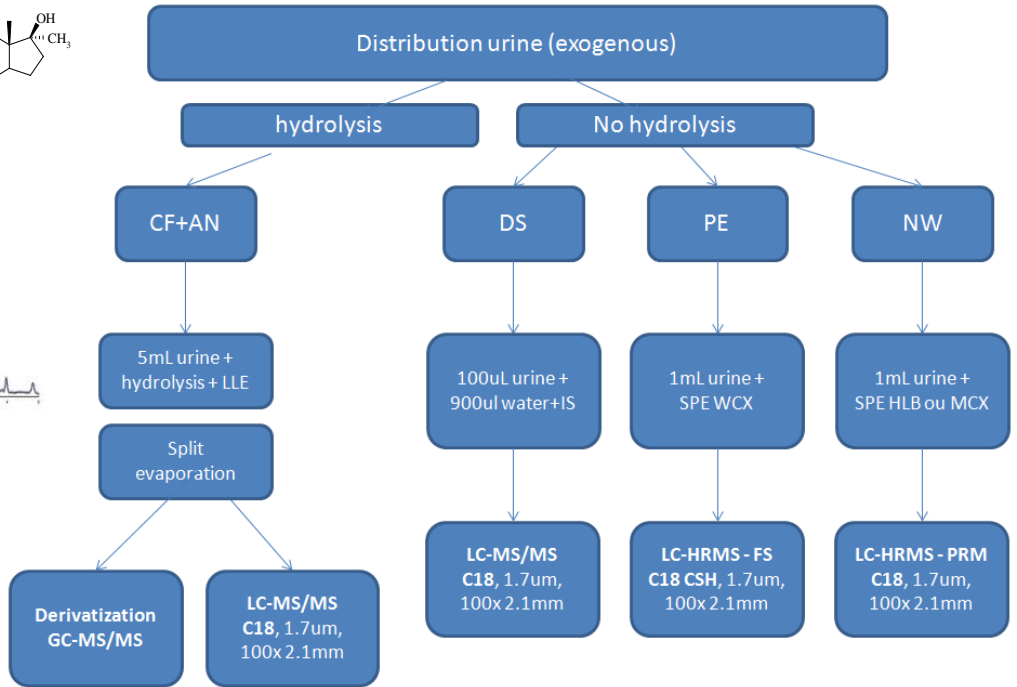
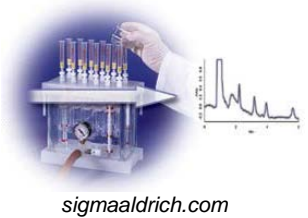
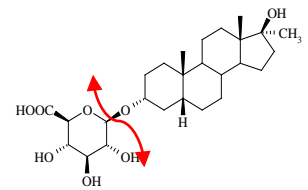
Metabolism – Phase-I



Metabolism – Phase-II



Analytical procedures – few tests, wide coverage



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Analytical procedures – criteria and limitations



5.2.4.3.1.2 Mass spectrometry (MS) coupled to either gas (GC) or liquid chromatography (LC) is the analytical technique of choice for confirmation of *Prohibited Substances*, *Metabolite(s)* of *Prohibited Substance(s)*, or *Marker(s)* of the *Use of a Prohibited Substance* or *Prohibited Method*. GC or High Performance Liquid Chromatography (HPLC) coupled with MS or MS-MS are acceptable for both Initial Testing Procedures and Confirmation Procedures for a specific analyte.



Additional tests e.g. GHRFs, IGF-1 analogs, insulins and GC/C/IRMS

5.2.4.3.1.3 Affinity Binding Assays (e.g. Immunoassays) are also routinely used for detection of macromolecules in urine samples. Affinity Binding Assays applied for the Initial Testing Procedures and Confirmation Procedures shall use affinity reagents (e.g. antibodies) recognizing different epitopes of the macromolecule analyzed, unless a purification or separation method is used prior to application of the Affinity Binding Assay to eliminate the potential of cross-reactivity. The Laboratory shall document, as part of the method validation, the Fitness-for-purpose of any such purification or separation method.



Additional tests e.g. ESAs, GH

Atypical passport finding – data review

➤ Is the sample valid?

- **Blood stability score, microbial contamination**
- Confounding factors (CF) and adverse analytical findings (AAF)
 - **Contact the laboratory for further information**



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➤ Are the data correct?

- Analytical data introduced to correct sample (laboratory)
- Sample assigned to correct BPID (SCA/TA)
 - **Steroid profile confirmation procedure**



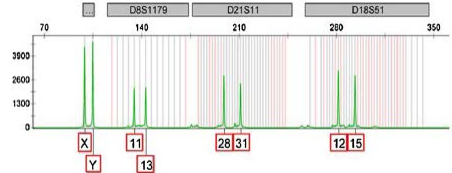
➤ What has been already done?

- Which samples were collected?
- Test menus requested per each sample matrix?
 - **Request the sample to be transferred to long-term storage**



Atypical passport finding – need of additional tests

- What is the scenario?
 - Sharing of information with SCA/DCO and laboratory; I&I
 - Data not available for passport evaluation - manipulated sample
 - Use of prohibited substance(s) - additional tests needed for identification



- Manipulated sample - testing for sample integrity and identity
 - Urine integrity:
 - **pH, specific gravity, steroid profile (included in the routine test menu)**
 - **creatinine, salt analysis, total protein, proteases (outside of routine testing)**
 - Sample identity
 - **DNA-analysis requires a reference sample (outside of routine testing)**

Int J Leg Med (2016) DOI 10.1007/s00414-016-1616-6 **Research article** **Research article** Drug Testing and Analysis
 ORIGINAL ARTICLE Received: 19 May 2014 Revised: 23 January 2015 Received: 3 October 2016 Revised: 21 October 2016 Accepted: 21 October 2016 Published online in Wiley Online Library: 18 November 2016

➤ Use of prohibited substance(s)

V. Castella · N. Di N. Robinson · M. **Determining the autDNA typing for personal identification of urine doping control by D after long-term preservation for testing in forensic identification of urine sample on enhancement of oxygen transfer (S2.1 and M1) nuclear and mitochondrial DNA marker doping control**

Kimiko Aoki,^{a,b,*} Hiroyuki Tanaka^a and Makoto Ueki^a



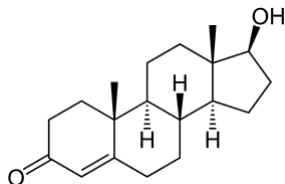
Additional tests for steroid module – S1.1b

➤ Steroid profile confirmation procedure

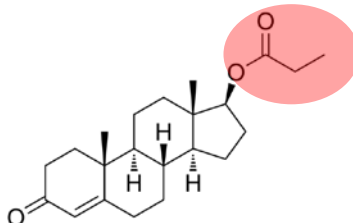
- GC-MS-analysis: repeated analysis of **steroid profile** parameters
 - Deeper insight also to microbial contamination
- **GC/C/IRMS**-analysis: exogenous/endogenous origin of testosterone metabolites



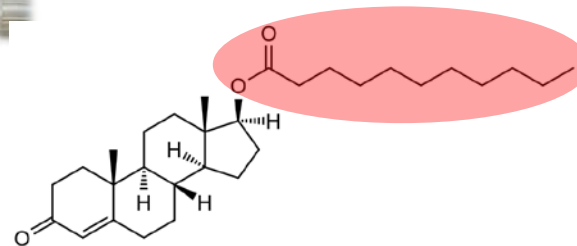
➤ Testosterone esters in **blood**



Testosterone



Testosterone **propionate**



Testosterone **undecanoate**



➤ Long-term AAS metabolites

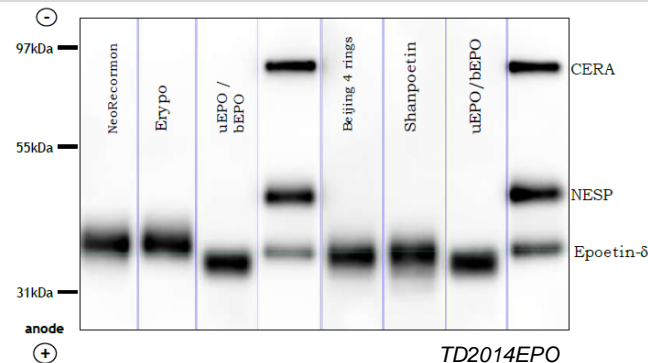
- For suppressed steroid profiles
- Direct analysis of glucuronide- and sulpho-conjugates



Additional tests for hematological module – S2.1 / M1

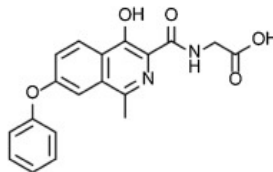
➤ Erythropoiesis stimulating agents (ESAs)

- **SAR-PAGE-based methods**
- Limited number of specific MS-methods (e.g. Hematide)
- Selection of matrix; **serum** often better (e.g. CERA)



➤ Agents activating hypoxia-inducible factor (HIF)

- **Molidustat, roxadustat (FG-4592)**
- Cobalt
- Xenon



➤ Detection of blood transfusions

- **Phthalates by MS-methods** in **urine**
- Flow cytometric analysis for homologous transfusions
- DNA-analyses for mixed populations



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Conclusions

- Routine analytical testing

- Preference of mass spectrometric methods to detected relevant target compounds
- Arranged according to instrumentation and capacity by each laboratory
- Wide coverage, but specific methods applied only upon additional analysis requests



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- Atypical passport profile – data review

- Verify the correctness of analytical data
- Validate the connection between the results and appropriate BPID
- Check the availability of various sample matrices collected in the test

- Additional tests

- Scenario: sample manipulation or use of prohibited substances?
- Steroid module: S1.1b substances, Hematological module: S2.1 and M1
- Discuss with the laboratory, **transfer the sample for long term storage**



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- **Dr. Hans Geyer (Cologne)**
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