PROJECT REVIEW

"Application of Time-of-Flight mass spectrometry for the unification and expansion of the window of screening methods of the WADA laboratories."

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The aims of the proposed research study are the following:

- Unification of the WADA Accredited Doping Control Laboratories screening procedures of classes of prohibited substances including classical small molecules like stimulants, narcotics, steroid agents, diuretics, etc, in order to free laboratory resources to new classes of prohibited substances: e.g. proteins. Modern sensitive high mass resolution, full mass range fast scanning mass spectrometer technology Time-Of-Flight, coupled with GC and LC chromatographic systems, capable to cover all classes of small molecules prohibited substances in minimum analytical runs per samples will be used. Sample preparation techniques using the minimum of steps but compatible with all classes of small molecules/prohibited substances will also be used. Incorporation of new drugs or metabolites, belonging to similar classes of molecules, with minimum or no changes to the laboratory preparative/analytical methods will be facilitated.

- Development of two new analytical tools, based on the instrumentation referred to in the previous paragraph, in order to widen the spectrum of the detection of prohibited substances or metabolites that do not exist as reference materials in the laboratory: a) creation of predictive analytical data, for molecules that are computer designed in the laboratory, b) bioassay-directed identification of unknown molecules

"Membrane Assisted Isoform Immunoassay (MAIIA I) a unique method for rapid detection of rhEPO in doping"

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Results and Conclusions

A novel, rapid and easy-to-use method, EPO WGA MAIIA, for determination of aberrant EPO isoform subpopulations in urine or serum, has been tested for its use as an EPO doping control method. The method separates EPO subpopulations due to their different interactions with the lectin wheat germ agglutinin (WGA). The glycosylated structures on recombinant epoetins show stronger interaction with the lectin, probably due to their higher content of polylactose amine. The WGA-based separation of isoforms and the subsequent ultrasensitive EPO determination is rapidly carried out within a few square cm of a thin porous layer formed as a test strip, using an image scanner for quantification. The test takes only 20 min. to perform and is well suited both for determination of single samples and for large series.

Before analysis with the EPO WGA MAIIA method, EPO is purified and concentrated from urine or plasma by use of a newly developed disposable EPO affinity purification device (www.maiiadiagnostics.com). With this easy-to-use sample preparation device EPO can rapidly be captured from large sample volumes and be eluted in a final volume of only 55 µL. The high EPO recovery of 65%, and the retained isoform distribution, makes the device a useful pre-step tool also for e.g. IEF, SDS-PAGE and LC/MS.

The EPO WGA MAIIA method allows detection of recombinant EPO in urine specimens from patients up to about 7 days after the last injection ($p<0.0001$). Recombinant epoetin e.g. alpha, beta, omega, delta, zeta and four Chinese types ($p<0.0001$), and EPO analogues like Aranesp ($p<0.0001$) and Mircera can be distinguished from endogenous EPO isoforms. Mircera shows less interaction with WGA compared to endogenous EPO, while recombinant EPO:s show stronger and Aranesp shows the strongest interaction. Only 2 pg of EPO is required for isoform detection, which is about 1/10 of the amount of EPO required for the presently used IEF based doping method. When rhEPO beta and endogenous EPO appear in the same sample it is possible to detect rhEPO down to a level where it constitutes only 40% of total EPO.

Besides measuring the interaction of the various types of EPO with WGA, it is possible in the same test strip to utilize also their interaction with the anti-EPO immobilized in the detection zone (see J. Immunol. Meth. 339 (2008) 236–244). By interpreting the antibody interaction profile, using the EPO AbQ MAIIA algorithm, it is possible to distinguish EPO and epoetins from EPO analogues like Aranesp and Mircera. The EPO WGA MAIIA test gives also an estimate of the EPO concentration in the eluate, enabling calculation of optimal application volume for the IEF or SDS-PAGE confirmation test.

The recommended test set-up for doping control utilizes EPO WGA MAIIA for identification of epoetins and Aranesp, while Mircera preferably is identified by EPO AbQ MAIIA.

The EPO WGA MAIIA test classifies all tested epoetin varieties and EPO analogues correctly, shows good resolution between endogenous EPO and epoetins, and can measure very low amounts of EPO. The quality controlled reagents will be supplied world-wide as a complete kit. The hands-on time is reduced compared to presently accredited tests, which significantly decreases the analysis cost. The excellent results and the easy-to-use concept seem to fulfil the requirements for a screening EPO doping control test.

Such a test makes it possible to considerable increase the number of EPO doping controls performed without increasing the total analysis cost.