PROJECT REVIEW

"Novel Biomarkers in Recombinant Human Growth Hormone Detection. LC-MRM-MS Method for Fibronectin Quantification in Anti-Doping Routine"

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The World Anti-Doping Agency (WADA) establishes two procedures to detect recombinant human growth hormone abuse (rhGH) in sports. The first, the "hGH Isoforms Test", is based on the separate measurement of growth hormone (GH) isoforms using immunoassays. The second, the "hGH Biomarkers Test", involves the analysis of two GH-responsive proteins, IGF-1 and P-III-NP, using either immunoassays and/or mass spectrometry (MS)-based techniques. Although being currently applied in routine analysis in anti-doping laboratories, both present some limitations. Main drawback of the "hGH isoforms Test" is the short window of opportunity whereas for the "hGH biomarkers Test" is their dependence of age and sex parameters and the more rapid elimination of IGF-1 compared to P-III-NP reducing retrospectivity.

Recent studies have described fibronectin (FN) as a long-term biomarker of rhGH abuse. These results have been obtained applying enzyme-linked immunoassays (ELISA) to serum and dried blood spots (DBS). Thus, the inclusion of FN in the evaluation of potential rhGH misuse would improve detection of cheating athletes. However, immunological tests suffer from some limitations that MS-based methodologies could overpass. This project aims to develop and validate a liquid chromatography multiple reaction monitoring mass spectrometry (LC-MRM-MS) method to measure FN concentrations in serum and DBS. The potential advantages of the MS-based methodology will be assessed and special focus will be done on pre-analytical effects of storage conditions and the introduction of freeze-thaw cycles. Preliminary results from our laboratory show that FN determination is affected by these pre-analytical effects. The new method will be used to quantify FN in samples from an administration study with subjects treated with rhGH and in a control group of healthy subjects to obtain preliminary reference values and preliminary potential decision limits.