PROJECT REVIEW

"Enhancing the sensitivity of the 2D-PAGE detection assay for hGH doping – follow-up-study"

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The misuse of recombinant growth hormone in elite sports is well known from confiscations and confessions and additionally, the first two positive samples were reported in 2009 using the luminescence immunoassay developed by Bidlingmaier and Strasburger. The luminescence immunoassay (LIA) provides a powerful screening tool but a complementary method for confirmation providing more detailed information would be desirable. Therefore, a method based on immunoaffinity purification, 2D-PAGE and immunblotting was developed which detects discrete endogenous variants of growth hormone. After successful development and validation, the methods´ sensitivity and robustness need to be optimized.

The project is planned to improve the sensitivity to be similar to that reached by the LIA to yield a powerful confirmation method. This can be done by optimizing a) the immunoaffinity purification e.g. by coupling specific antibodies directly to magnetic beads, b) the blotting conditions or the immunodetection, e.g. by using different secondary antibodies for the visualization and detection. Furthermore, the robustness of the method should be improved by providing another primary antibody which could replace the currently used one to ensure continuous availability.

After optimization, another follow-up project could include the measurement of a reference population to allow the calculation of reference values.

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Results and Conclusions:

The aim of this project was to enhance the sensitivity of the existing 2D-PAGE detection assay for hGH doping by optimizing different steps of the sample preparation protocol. As the GeneTex antibody currently used within this confirmation method is not produced anymore, it was of exceptional urgency to find an alternative antibody with adequate binding properties and providing the desired sensitivity. Out of four tested antibodies, one proved to be an adequate alternative as it was not only able to detect rGH amounts down to 0.25 ng but also to bind all endogenous variants of hGH. Thus, the antibody was subjected to protein.

A purification and the sample preparation protocol was optimized by testing different antibody concentrations, incubation times and secondary antibodies. Finally, it was successfully implemented into the whole confirmation procedure comprising immunoaffinity purification, 2D-PAGE and immunoblotting (with additionally incorporated secondary antibody amplification). Due to these new aspects and requirements of the methodology, further evaluation of the performance and applicability to authentic administration study samples might be required. Due to the necessity of finding a primary antibody serving as an alternative to the currently (though discontinued product by GeneTex), the experiments concerning the immobilization of a primary antibody to magnetic nanoparticles) could not performed within this project as originally planned. The incidence however underlines once more the importance of a continuous supply (and assurance) of consumables for doping control analytical assays, which is hardly guaranteed with polyclonal antibodies. In the absence of technical alternatives (e.g. MS-based methodologies), the use of monoclonal antibodies (as employed in the LIA test system) might be preferable to ensure constant quality and comparability of analytical results.