PROJECT REVIEW

"Nanoparticles-based immunomagnetic extraction-capillary zone electrophoresis-native fluorescence detection: a novel strategy towards fast and cost-efficient detection of erythropoietin"

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The current strategies used for the detection of EPO in sports are labour-intensive, time-consuming and cost-inefficient. The aim of this project is to develop an innovative strategy towards a fast and cost-efficient EPO assay in sports. This strategy is based on two key steps: 1) nanoparticles-based immunomagnetic extraction (NPIME), and 2) capillary zone electrophoresis (CZE) separation coupled with on-line sample concentration (OLSC) and native fluorescence (NF) detection.
First, a NPIME approach will be developed. Using anti-EPO antibody immobilized magnetic-cored nanoparticles, recombinant and natural EPO in samples is specifically extracted onto the nanoparticles and then desorbed into a much smaller volume. This process effectively concentrates EPO and meanwhile eliminates interference from the sample matrix. Second, a CZE-OLSC-NF method will be established. While the CZE separation provides fast profiling of EPO glycoforms, distinguishing recombinant and natural EPO. The OLSC allows for concentrating EPO from a larger injected sample volume into a much narrower zone before separation, thus significantly reduces the detectable concentration level. The NF detection selectively detects EPO with high sensitivity. Finally, the developed NPIME approach and the CZE-OLSC-NF method will be combined in off-line mode and applied to EPO spiked urine or urine samples of EPO injected patients. The proposed strategy can provide two significant advantages: speed and overall cost-efficiency. Our goal is to reach a total analysis time of less than 4 hours and an overall limit of detection (LOD) of 10-11 M at the end of the project proposed. The overall performance can be further effectively improved by in-depth optimization of the individual techniques. Also, the sensitivity can be further significantly improved by using a deep UV laser as the excitation light source. Thus the strategy proposed can be eventually developed into a reliable and robust analytical method for anti-doping analysis of EPO in the near future.

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Results & Conclusions:

The current EPO assay is associated with apparent drawbacks: labour-intensive, time-consuming and cost-inefficiency. This assay involves four key component methods: 1) isoelectric focusing (IEF), 2) ultrafiltration, 3) double blotting, and 4) on-gel chemiluminescence detection. The four key component methods are essential for specific and sensitive detection of EPO; however, they are all labour-intensive and time-consuming.
The aim of the project is to explore straightforward and effective substitutes for these sub-methods and then integrate them into a fast and cost-efficient approach.

Nanoparticles (or microbeads)-based immunomagnetic extraction (NPIME or MBIME) is used as a replacement of ultrafiltration and double blotting. Capillary zone electrophoresis (CZE) is used as a substitute of IEF and the combination of native fluorescence (NF) and on-line sample concentration (OLSC) is used as an alternative for the on-gel chemiluminescence detection. We have successfully synthesized immunoaffinity magnetic nanoparticles (MNPs) using anti-alpha fetoprotein IgG as a model antibody. However, when the established method was transferred to anti-EPO antibody, the obtained immune-MNPs failed to recognize EPO. The reason for this seems that the antibody used was too susceptible to the reaction conditions for the immobilization. To overcome this issue, we turned to commercial protein A-coated magnetic beads and developed MBIME approach for specific extraction of EPO, which could enrich EPO by one order of magnitude. Besides, we successfully established an in-lab built deep UV laser-induced fluorescence (deep UV-LIF) detection system for detection of the native fluorescence of EPO. It enhanced the detection sensitivity by one order of magnitude as compared with UV absorbance detection. CZE was an effective replacement of IEF, which permitted complete resolution of EPO glycoforms within 25 min.

OLSC could enhance the detection sensitivity by 50-100 fold, however, when used for the sample prepared by the MBIME approach, it resulted in notable loss in separation resolution. So it was excluded as a sub-method for the integration. Based on the component approaches established, the finally integrated approach, MBIME-CZE-deep UV LIF, allowed for the extraction, separation and detection of EPO glycoforms within 6.5 hours. However, the detection sensitivity was enhanced only by two orders of magnitude, which is far from the requirement for the analysis of real samples. To further develop the proposed strategy into practical approach for real sample assay, solutions to overcome the experienced issues were found. As human urine exhibited very significant matrix effect, antibody without cross reactivity to other proteins is a critical requirement for specific extraction. Besides,

high binding affinity is a critical requirement for more efficient extraction. Furthermore, inertness under immobilization conditions is essential for the synthesis of active immune-affinity MNPs. An anti-EPO antibody that can meet these requirements is the key for the practical use of this approach.