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## **Towards the screening of urinary EPO by SDS-PAGE analysis**

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### **Abstract**

The method currently used in the antidoping laboratories for the detection of urinary erythropoietin (EPO) is based on the charge differences observed between endogenously produced EPO (huEPO) and recombinant EPO (rhEPO) when analysed by isoelectric focusing (IEF). Previous reports have demonstrated that by SDS-PAGE, the different EPO molecules also possess a different apparent molecular weight (MW). The aim of this study was to determine if this size difference could be used as a discrimination factor between the and lead to the development of a simpler screening procedure for the analysis of urinary EPO. By SDS-PAGE, results show that huEPO presents an apparent molecular weight of 36 kDa, rhEPO of 38 kDa and NESP of 49 kDa. In order to lower the protein concentration prior loading on gel, an immunoaffinity procedure was developed for the specific extraction of EPO from urine samples. The results indicate that it is possible to extract EPO from urine samples and to obtain a similar sensibility level than the IEF method. Moreover, the method has the potential to determine which type of EPO is present in a sample, as observed from the analysis of negative, positive or spiked urines. It was also shown that the detection of rhEPO presence in a sample can be achieved when it represents the third and more of its total EPO content. Importantly, with this method, no interference from effort-like or active samples was observed in the rhEPO detection region, as it is currently occurring with the IEF method. It is proposed that the SDS-PAGE method could be used as a first screening method for the analysis of urinary EPO, in complement to the IEF method for the confirmation analysis.