**PROJECT REVIEW**

## *"Reliable assay for rapid detection of potentially unknown erythropoietin mimetics chemically unrelated to endogenous hormone in biological samples"*

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Recombinant erythropoietins (EPOs) have long been one of the most frequently abused drugs for increasing blood oxygen capacity. However, numerous compounds with EPO-like activity but completely different chemical structures (either short peptides or even nonpeptidic synthetic molecules) have been developed so far and due to such chemical diversity, detection of their potential abuse is notoriously difficult. To our knowledge, no universal and reliable analytical method is available to confirm the presence of all EPO mimetics in biological sample regardless of their chemical structure. Here, we describe development of a rapid and universal assay for detection of any ligand binding to the erythropoietin receptor (EpoR) which could be used as a low-cost screening test in doping control to identify individuals for further detailed examination. Our innovative platform comprises combination of immunoprecipitation steps and two modified ELISA tests where recombinant filamentous phage particles displaying EpoR are used instead of primary antibodies. As evaluated with spiked artificial urine, such assay can detect the presence of EpoR ligands chemically unrelated to EPO in specimens. Site-specific proteolytic cleavage and isolation of EpoR:ligand complex from phage particles enables analysis of the complex by mass spectrometry and potential identification of previously unknown EpoR ligand. The described platform may also be further developed into the lateral flow immunoassay format similar to common over-the-counter pregnancy test which would greatly increase its applicability out of the laboratory. To emphasize, such concept can also be applied to detection and identification of other growth factor or hormone mimetics by implementation of appropriate combination of receptor, ligands and antibodies. Moreover, based on our platform a multiplex assay capable of simultaneous detection of substances with distinct activity can be developed by utilizing Luminex® technology and appropriate combination of recombinant phage particles displaying different hormone and growth factor receptors.