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Detection of Recombinant Erythropoietin in Urine by Two-dimensional Gel  
Electrophoresis

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## **Detection of recombinant erythropoietin in urine by two-dimensional gel electrophoresis**

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### **Summary**

The International Olympic committee banned the use of recombinant human erythropoietin (rHuEPO) in 1990. Since then there has been a growing need to develop a sensitive, reproducible and less complex technique than the currently used method to identify rHuEPO. The world anti-doping agency (WADA) has evaluated the current EPO test and recommended several areas [1] to improve the test [2-4]. The areas include: (a) pre-assessment of urine samples prior to sample preparation; (b) selective urine pre-concentration; (c) controlled solubilization of the urine concentrate using multiple chaotropic agents and CHAPS detergent; (d) improved electrophoretic separation using immobiline pH gradient (IPG) strips; (e) use of more appropriate blotting membranes and antibodies; (f) recent luminescence kits and (g) a new approach for the interpretation of the scanned EPO profiles.

We present a two-dimensional electrophoresis (2DE) method for the detection of endogenous human erythropoietin (HuEPO) and rHuEPO from urine that is cost effective, reproducible and more accurate than the currently used one-dimensional (1D) isoelectric focusing (IEF) test, which involves double blotting [4]. This 2DE method addresses all the areas recommended by WADA, and involves a simple and high throughput urine preparation, single blotting and separates HuEPO and rHuEPO distinctly by iso-electric point (pI) and molecular mass.

We identified that the monoclonal EPO antibody used in the current 1D test is cross-reactive to at least four major urinary proteins; Tamm Horsfall glycoprotein, alpha-antichymotrypsin, alpha-2-thiol proteinase inhibitor and alpha-2-HS glycoprotein separated on pH 3 – 5, which has not been reported earlier. The pI of these cross-reactive proteins overlaps with HuEPO and rHuEPO, but they separate distinctly by molecular mass. This physical separation of EPO (both HuEPO and rHuEPO) and cross-reactive urinary proteins is

not possible if separated by 1D IEF only. Furthermore, an internal standard has been incorporated into the 2DE protocol, which is detected using the same Western detection system as EPO. The internal standard separates within the same pI range of EPO but with a higher molecular mass that facilitates an accurate identification of rHuEPO by image analysis. Software, (EplQ), specific for the detection of rHuEPO, analyzes the 2DE image accurately and automatically.

The combination of sample preparation, two-dimensional separation of EPO from urinary proteins on narrow pH range gels, internal standard, standardized blotting procedures and image analysis software should enable the test for rHuEPO drug to be performed reproducibly by different laboratories around the world. This will provide an accurate detection and analysis of urinary rHuEPO.

An initial blind trial analysing 23 samples showed that the criteria used to assess the gels needed to be improved as there appeared to be 2 false positive results. Using the 2DE method we carried out a further analysis of 21 spiked urine samples (blinded) supplied by National Measurement Institute (NMI) in Sydney each of which were analysed and scored using the current 1D protocol. The analysis using 2DE resulted in one false negative of the rHuEPO drug in the urine. No false positives have been obtained since the image analysis software has been developed and used with the addition of an internal standard. Further samples are being trialled in collaboration with the NMI in order to continue validation the test.

## References

- [1] Peltre, G. & Thormann, W. (2003) Evaluation report of the urine EPO test. *Council of the World Anti-Doping Agency (WADA)* Paris and Bern, March 11.
- [2] Lasne, F. & de Ceaurriz, J. (2000) Recombinant erythropoietin in urine. *Nature*, 405, 635.
- [3] Lasne, F., Martin, L., Crepin, N. & de Ceaurriz, J. (2002) Detection of isoelectric profiles of erythropoietin in urine: differentiation of natural and administered recombinant hormones. *Anal. Biochem.* 311, 119-126.
- [4] Lasne, F. (2001) Double-blotting: a solution to the problem of non-specific binding of secondary antibodies in immunoblotting procedures. *J. Immunol. Methods* 253, 125-31.

The summary is based on an article (Title: “Improved urinary EPO drug testing using two-dimensional gel electrophoresis”) submitted for publication in *Nature Biotechnology*.