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Validation and implementation of MAIIA columns into routine analysis of urinary EPO screening

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Abstract

MAIIA Diagnostics have developed an EPO purification kit, using immunoaffinity columns (IAC), for rapid purification and concentration of endogenous or recombinant EPO (rhEPO) from biological media such as urine and serum. This was used as a pre-step for EPO analysis by isoelectric focusing (IEF) and double Western blotting techniques. This method was compared to the current ultrafiltration (UF) method employed in ASDTL. Data from a recent administration study where subjects were given Eprex® or Aranesp® were analysed.

Comparison of IEF profiles of blank urines from both sample preparations showed improvement in isoform profiles and reduction in band smearing using the IAC preparation method. Bands in the basic region associated with zinc-alpha-2-glycoprotein (ZAG) by UF preparation were not observed in the samples that were purified by IAC. IEF analysis of Eprex® and Aranesp® administration samples indicated better isoform profiles using IAC preparation. Interestingly, all NESP samples, from both UF and IAC sample preparation, failed the third criteria of TD2009EPO [5].

Introduction

EPO is a glycoprotein hormone produced by the kidney in response to low oxygen tension in the blood. rhEPO is used legitimately to improve erythropoiesis in anaemia due to kidney failure and in anephric individuals [1]. rhEPO is banned by WADA and has been prohibited for more than two decades.

Lasne et al [2] developed the current EPO method using IEF/double Western blotting to separate and identify the isoforms of EPO [3]. This method has largely remained unchanged since its development. Swedish company MAIIA Diagnostics have developed IAC to aid in the concentration and purification of EPO in urine, serum and plasma samples.

This study aims to compare IAC purification to UF sample preparation to determine the effectiveness for routine analysis of urine samples.

Experimental

Initial comparison testing of IAC and UF consisted of blank urines from different subjects. An administration study was also conducted where 2 female volunteers were administered single doses of Eprex® (9600 IU as single dose) or Aranesp® (220 mcg as single dose). Samples (blood and urine) were collected daily for up to 5 days. All samples prepared from both IAC and UF methods were submitted to IEF analysis. All result interpretation was conducted using GASepo v2.1 software and positivity criteria were based on the current technical document for EPO (TD2009EPO v2). [5]

Results and Discussion

In general, isoform profiles improved using IAC purification (Figure 1). In sample 1, the UF prepared sample showed 'burn out' after exposure due to overloading compared to IAC prepared sample, which may be attributed to IAC loading efficiency.

A reduction in band smearing was observed with IAC purification compared to UF concentration, while increasing band straightness (sample 4, Figure 1). Samples purified by IAC did not have bands associated with ZAG [4] as observed in UF prepared samples 1, 2 and 4. With sample 3, low EPO levels were observed in both preparation methods but the IAC sample had better band straightness.

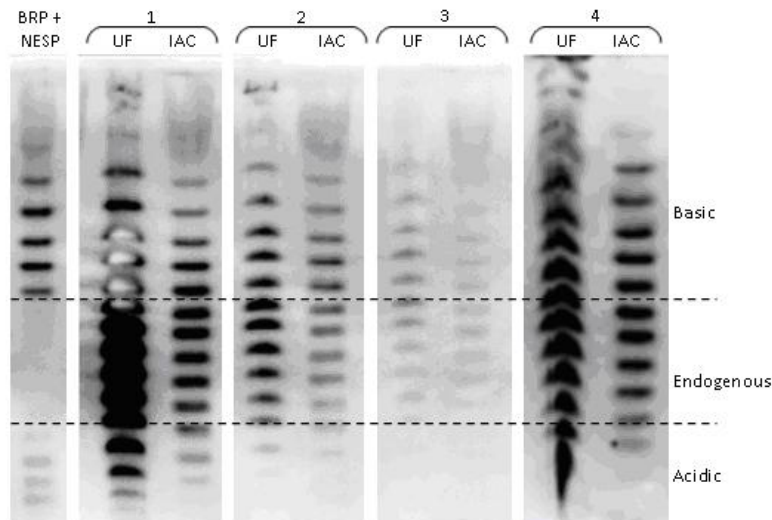


Figure 1. IEF gel displaying retentates and eluates prepared from blank urine samples 1, 2, 3 and 4 by UF and IAC respectively. Positive controls BRP and NESP.

ADMINISTRATION STUDY

Results shown in Figure 2 (single Aranesp® dose) clearly indicated NESP in the samples. However, the percent acidic isoforms (PAI) were generally higher in the UF prepared samples. The lower affinity of the IAC for NESP was observed and has also been shown by others [6].

Interestingly, no sample from either UF or IAC sample preparation met criteria 3 of TD2009EPO [5] (3.2.3 - Darbepoetin alpha), with band 'D' being the least intense out of the 3 bands.

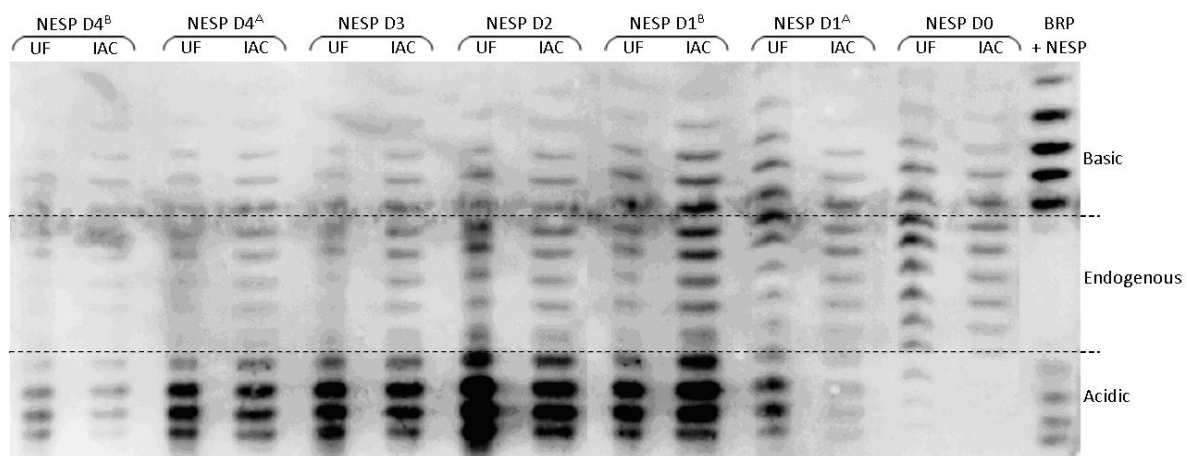


Figure 2. IEF gel displaying retentates and eluates of an Aranesp excretion study. Urine samples before injection (D0), and days 1-4 after injection (D1-D4) were prepared by UF and IAC. Positive controls BRP and NESP. Note: 2 urine collections made on days 1 and 4, denoted as D1A, D1B, D4A and D4B.

The subject administered Eprex®, had little endogenous EPO (D0, Figure 3) at baseline. After administration, samples appeared suspicious (D1-3), and when annotated, all met positivity criteria as in TD2009EPO [5].

While it appeared that samples purified by IAC had greater intensities, the PBI between the two preparation methods were not different. This may be due to differences in EPO concentration loaded as UF prepared samples were normally measured by ELISA (e.g. Immulite) and diluted to 500 IU/L on gel, where as IAC prepared samples were not measured and were loaded directly.

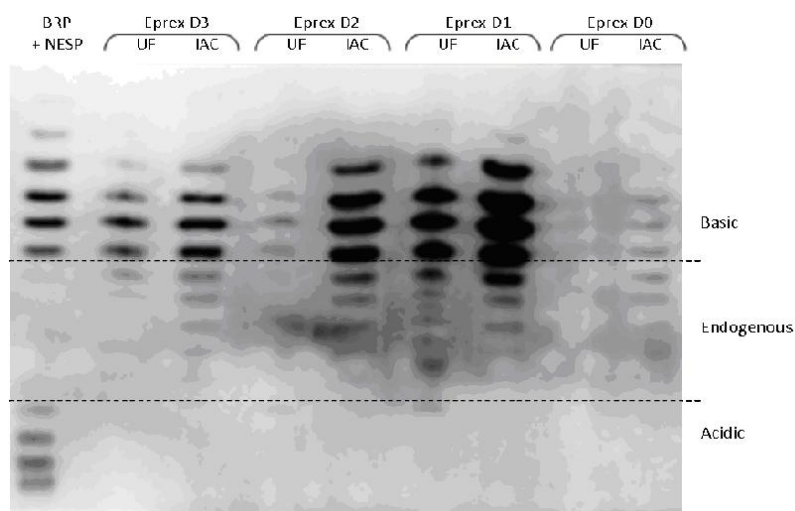


Figure. 3 IEF gel displaying retentates and eluates of an Eprex excretion study. Urine samples before injection (D0), and days 1-3 after injection (D1-D3) were prepared by UF and IAC. Positive controls BRP and NESP.

Conclusions

Preparation of urinary samples by IAC for EPO analysis showed considerable improvement in isoform quality, especially noticeable in samples that showed smearing or contained zinc-alpha-2-glycoprotein. Given the limited loading capacity of MAIIA columns, overloading on gels as well as isoform band overexposure of EPO were not observed in this study. Therefore, IAC sample preparation method was a viable, and now a cost effective, method for use in the analysis of EPO in routine testing.

References

1. Winearls C.G, Oliver D.O, Pippard M.J, Reid C, Downing M.R, Cotes P.M (1986) Effect of human erythropoietin derived from recombinant DNA on the anaemia of patients maintained by chronic haemodialysis. *Lancet* **328**,1175-1178.
2. Lasne F, de Ceaurriz (2000) Recombinant erythropoietin in urine. An artificial hormone taken to boost athletic performance can now be detected. *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. *Analytical Biochemistry* **311**,119-126.
4. Reichel, C (2008) Identification of zinc-alpha-2-glycoprotein binding to clone AE7A5 antihuman EPO antibody by means of nano-HPLC and high-resolution high-mass accuracy ESI-MS/MS. *J. Mass Spectrom* **43**, 916-923
5. WADA Technical Document - TD2009EPO <http://www.wada-ama.org/> (accessed Feb 2012).
6. Dehnes Y, Lamon S, Lönnberg M (2010) Erythropoietin (EPO) immunoaffinity columns - A powerful tool for purifying EPO and its recombinant analogues. *J. Pharmaceut. Biomed. Anal* **53**,1028-1032.