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Easy to use IEF compatible immunoaffinity purification of urine retentates.

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Extended Abstract

The effective Technical Document of the World Anti-Doping Agency (WADA), TD2009EPO, provides two electrophoretic methods for Erythropoietin (EPO) detection and discrimination in urine, namely isoelectric focussing (IEF) and SDS-PAGE. Routinely collected urine samples are concentrated 500 to 1000 fold, leading to high protein abundance in the retentates. Sample overloading due to high protein abundance can lead to streaking and curvatures of the detectable bands making densitometric measurements unreliable. Heparin and possibly anionic detergents from gloves that might be used during doping control have also been shown to disturb EPO isoform patterns during IEF. For the anti-EPO antibody cross-reactivity with a second urinary protein has been observed. Immunoaffinity purification of urine samples is a convenient way to reduce background, avoid cross-reactivity and purge the sample of IEF-interfering substances. A purification using ELISA wells has been successfully used prior to SDS-PAGE. This ELISA Kit was now used to purify samples with an IEF-compatible elution.

A significantly varying binding affinity of the anti-EPO antibody used in the ELISA wells to different EPO-isoforms needed to be excluded. Two sets of experiments were used to investigate if the ELISA purification ensures unchanged isoform distribution after purification. In the first approach buffer spiked with different epoetins were either directly applied to IEF or after immunoaffinity purification. None of the tested epoetins subjected to immunoaffinity purification showed an isoform distribution which differed from the distribution of the directly applied samples. In a second approach three different urine samples (blank urine, Silapo excretion study urine, Aranesp excretion study urine) were applied to IEF either purified or unpurified. Both excretion samples fulfilled the WADA

criteria of a positive sample prior to purification and still did afterwards. The blank urine remained negative after purification without an alteration of isoform distribution.

Recovery was determined using an EPO-ELISA from STEMCELL. The concentration of spiked buffers was measured before subjection to purification and afterwards. The purification showed recovery ratios between 50 and 90% depending on applied substance and application volume. It seemed that greater sample volumes applied to the ELISA wells lead to a reduced recovery. EPO bound to the upper parts of the well cannot be eluted efficiently. Optimal recoveries depend on effective concentration of urine retentates before purification.

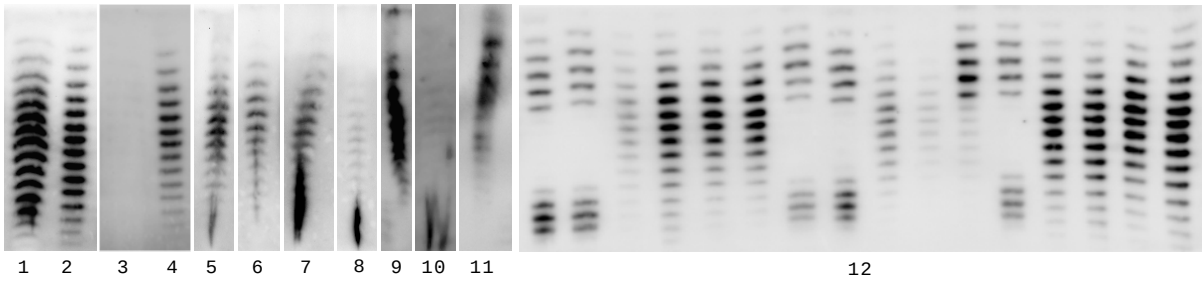

Fig. 1: Double-immunoblot after isoelectric focussing of doping control samples. Samples were either directly applied (1, 3, 5-11) to the gel or after immunoaffinity purification (2, 3, 10). 1-11 doping control samples; 12 typical double-immunoblot of a routine IEF screening gel since adoption of the purification (image includes lanes with doping control samples and lanes with a 12 mU BRP / 0.12 ng NESP Standard).

This method has been shown to be a valuable tool to improve gel quality.

Typical disturbances of the isoelectric pattern are shown in figure 1 (1, 3, 5-11). These can be band curvature (1), loss-of-signal (3), streaks (5-8), compression (9) or excessive background (11). The discussed disturbances have not been seen in routine screening gels since the adoption of the described purification method (12). High recoveries rely on an efficient concentration of urine. The purification is easy-to-use and not work-time consuming. The ELISA immunoaffinity purification is a very flexible way to purify samples. By changing the elution buffer samples can be applied to either IEF or SDS-PAGE. The demonstrated method can easily be used for both screening and conformational analysis of urine samples. It is a good addition to the WADA accredited IEF method for EPO detection.

For further details please refer to:

Reihlen *et al.* Easy to use IEF compatible immunoaffinity purification of urine retentates. (publication in preparation)