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Detection windows of rEPO using SAR- and IEF-PAGE

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Abstract

Misuse of erythropoietin in sports has been documented for many years now. The Technical Document of WADA currently in force (TD2013EPO) allows two electrophoretic methods for screening of urine samples for recombinant erythropoietin (rEPO). The Initial testing procedure can be performed either by sarcosyl polyacrylamide gel electrophoresis (SAR-PAGE) or by isoelectric focussing polyacrylamide gel electrophoresis (IEF-PAGE). Differing detection windows of SAR-PAGE and IEF-PAGE could lead to false negative findings. The detection windows of two rEPOs, Silapo® and Biopoin®, after a single subcutaneous injection of 50 UI/kg and respectively 12.5 UI/kg in urine using SAR- and IEF-PAGE were investigated. Silapo® application could be detected by both techniques up to the last collected sample, 90h after application. When applying a Biopoin® micro dosage the IEF-PAGE could fulfill the identification criteria of TD2013EPO until 90h after application. While the 96h sample still suspicious it could not meet the criteria. The SAR-PAGE was able to meet the identification criteria up to the 96h sample.

Introduction

In sports, Erythropoietin abuse is a well-documented phenomenon [1]. Currently the Technical Document (TD2013EPO) allows two methods for EPO screening of urine samples: SAR-PAGE and IEF-PAGE. Though both methods are based on electrophoretic separation they differ in the style of separation. The SAR-PAGE separates proteins by apparent molecular weight exploiting the fact that most EPO analogs exhibit a higher molecular weight than endogenous EPO. In contrast, the IEF-PAGE separates proteins by isoelectric point. This leads to a sample specific isoform profile. Samples containing rEPO can be identified by densitometric quantification. The aim of this study was to investigate the detection windows in urine of two rEPOs, Silapo® and Biopoin®, after a single subcutaneous injection of 50 IU/kg and respectively 12.5 IU/kg using SAR- and IEF-PAGE.

Experimental

Urine samples of the excretion study were collected from a male volunteer before and at different time points after subcutaneous application of 50 IU/kg Silapo® (collected until 90h after application) and respectively 12.5 IU/kg Biopoin® (collected until 96h after application). Sample preparation was performed via ultrafiltration [2] and samples were then immunopurified using ELISA-wells [3,4]. The purified samples were then analyzed either by IEF-PAGE [2] or SAR-PAGE [3]. Images of the immunoblots were analyzed using GASepo 2.1. After analysis the identification criteria of TD2013EPO were applied to determine the detection windows of rEPO after application for both SAR- and IEF-PAGE.

Results and Discussion

Application of 50IU/ kg Silapo® could be detected both by IEF- and SAR-PAGE up to the last collected sample. A detection of Silapo® could be verified up to 90h using both techniques (Fig. 1A, 1B, 1C). The data suggest both techniques to have a longer detection window. Micro-dosing of Biopoin® was detectable 96h after application using SAR-PAGE. Though this was last collected sample (Fig. 2A, 2C) the data suggest a longer detection window. This will need to be verified.

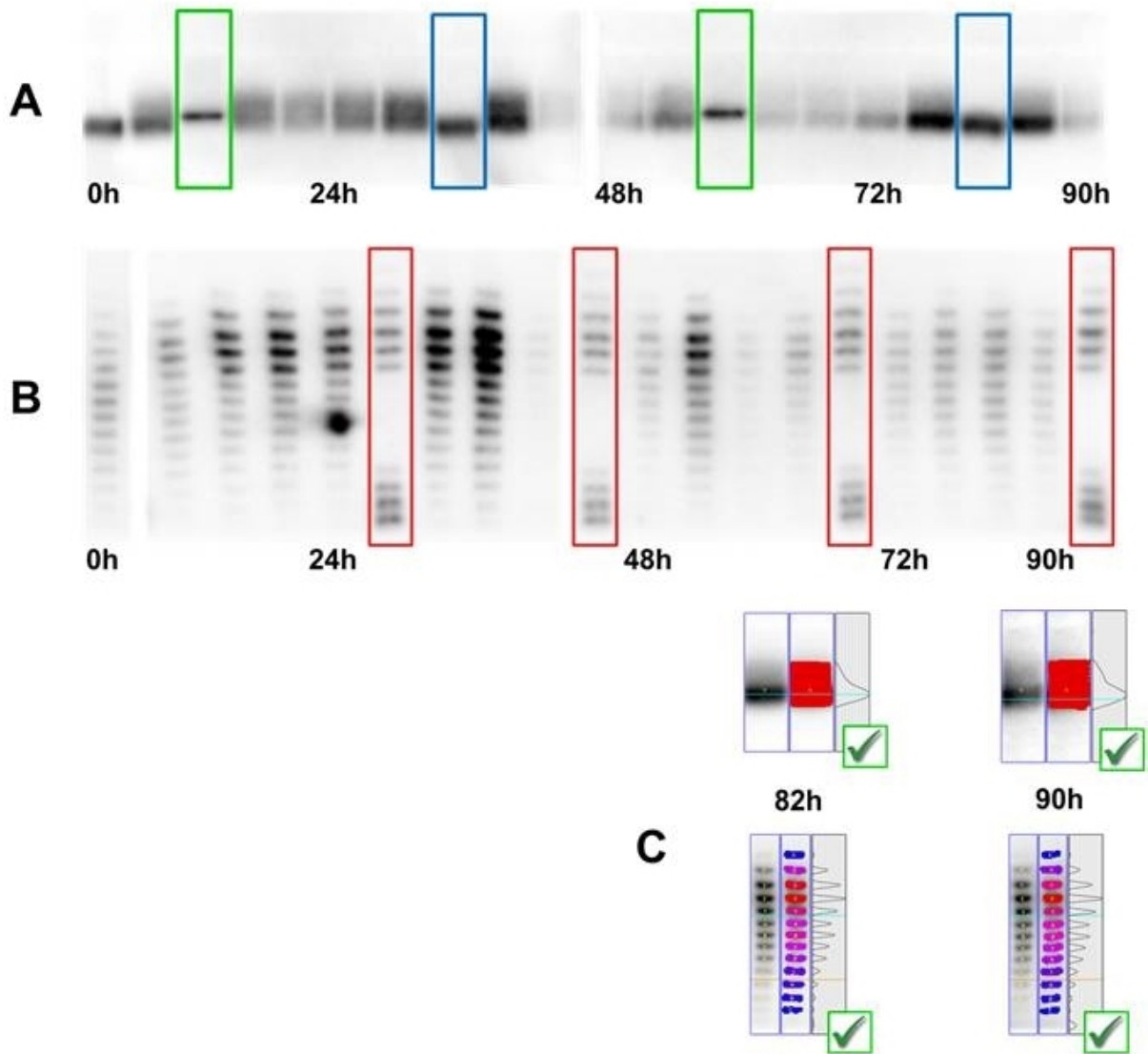


Fig 1: Immunoblots of urine samples after SAR-PAGE (A) and IEF-PAGE (B) showing Dynepo / NESP standard (green), urinary standard (blue), BRP / NESP Standard (red) and excretion study samples from before (0) to 90h after application of 50 IU/kg Silapo®. Modified GASepo report of the SAR- and IEF-PAGE analysis of the last two excretion study samples (C). A green tick marks samples which meet the identification criteria of the TD2013EPO.

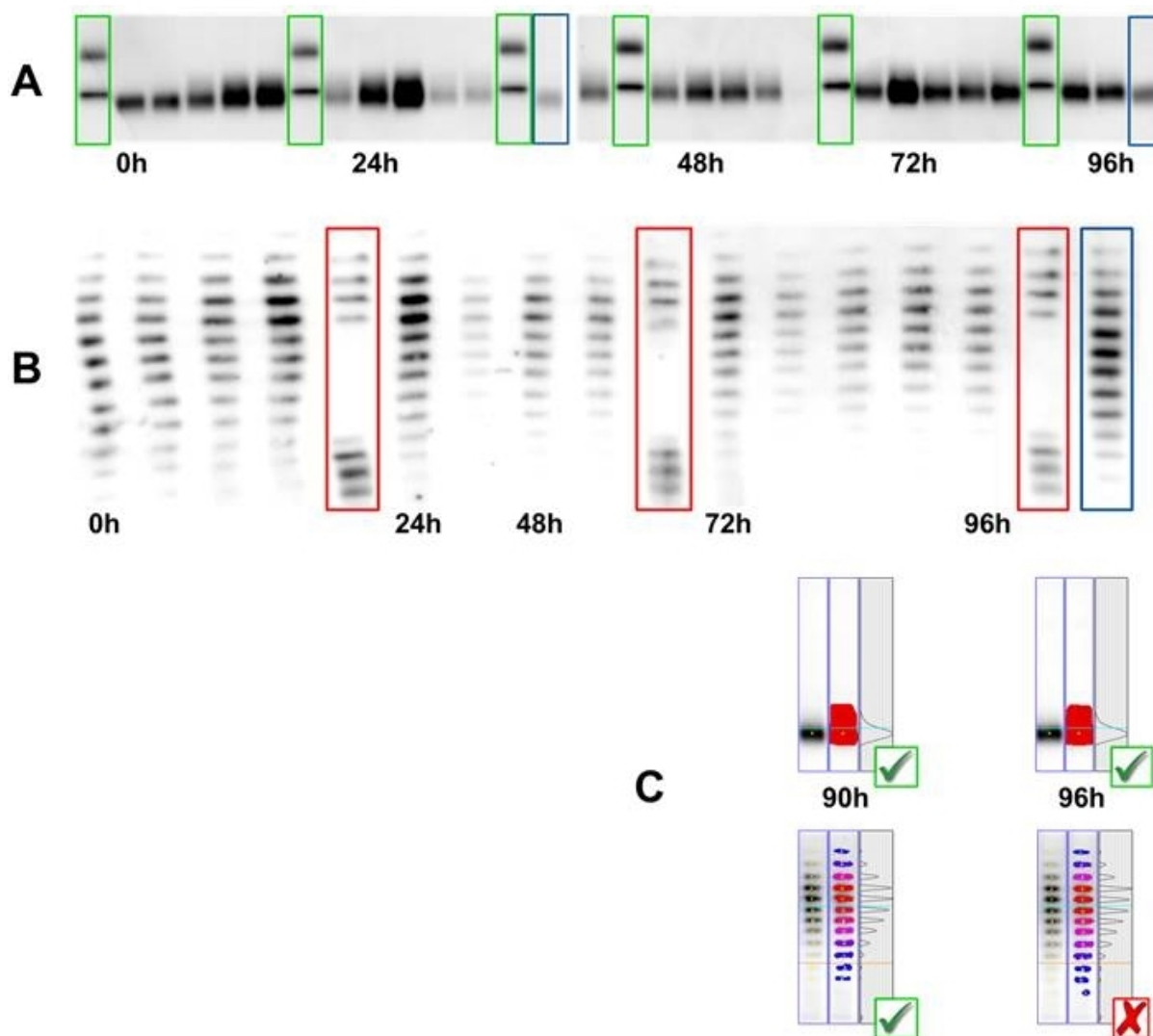


Fig 2: Immunoblots of urine samples after SAR-PAGE (A) and IEF-PAGE (B) showing Dynepo / NESP standard (green), urinary standard (blue), BRP / NESP Standard (red) and excretion study samples from before (0) to 96h after application of 12.5 IU/kg Biopoin®. Modified GASepo report of the SAR- and IEF-PAGE analysis of the last two excretion study samples (C). A green tick marks samples which meet the identification criteria of the TD2013EPO. A red cross marks those that do not meet the criteria.

With IEF-PAGE analysis it was possible to fulfill the identification criteria until 90h post application (Fig. 2B, 2C). Though the isoform pattern of the sample 96h after application was still suspicious it could not fulfill the criteria defined by the TD. The results for Biopoin® indicate a superior detection capability of the SAR-PAGE at later time points compared to IEF-analysis. Studies with prolonged sample collection times need to be conducted to further ascertain this conclusion. But it is already consistent with data from doping control samples analyzed in the Cologne laboratory which fulfilled the SAR-PAGE identification criteria but were unable to meet the identification criteria for IEF-PAGE.^[5] Following the guide line of the technical document this circumstance results in samples being reported as *Atypical* instead of *Adverse Analytical Findings*.

Conclusions

The data suggest that the SAR-PAGE has a longer detection window than the IEF-PAGE for the investigated EPO analogs. Therefore it should be the first choice for screening to avoid false negative samples.

References

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