

Pattern classification-derived criteria for the identification of epoetin- δ in urine

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INTRODUCTION

Epoetin- δ (DynepoTM) is the first erythropoiesis stimulating agent (ESA) to be derived from a human cell line. In March 2007, Shire Pharmaceuticals launched DynepoTM on the market of anemia treatment drugs. Like other ESAs, epoetin- δ was rapidly identified as a potent doping agent and misused by athletes trying to improve artificially their aerobic power and, consequently, their performance in endurance sport. Shire stopped the commercialization of DynepoTM end of 2008, but some copy molecules are already available on the market.

The isoelectric focusing (IEF) analysis of DynepoTM, that allows to distinguish between endogenous and exogenous EPOs, presents a urinary profile containing more acidic isoforms than the profiles of epoetin- α and - β . Consequently, epoetin- δ has an IEF pattern closer to the one of urinary endogenous EPO than classical recombinant EPOs have, what makes it harder to reveal. The current WADA identification criteria were notably designed for classical epoetins and, even if it has never been demonstrated formally, are probably poorly sensitive to detect DynepoTM.

EXPERIMENTAL PROCEDURE

Clinical Study

Samples collected from 6 healthy volunteers treated with 6 epoetin- δ injections (n=126) and from a control population (n=110) were immunopurified and analyzed with the usual IEF method [1].

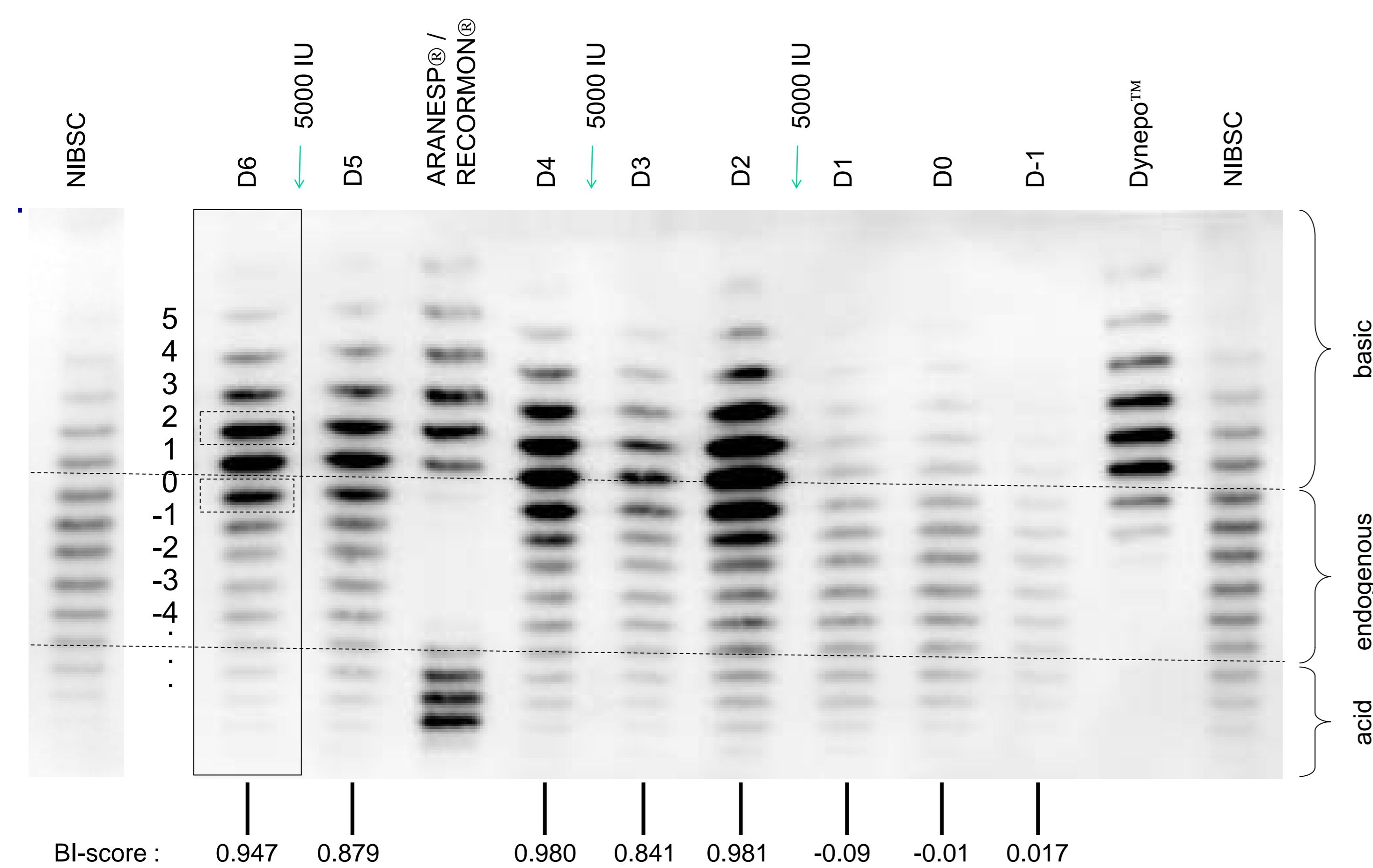


Figure 1: IEF gel showing the first urine samples of days -1 to day 6 for one subject, with usual positive controls (Aranesp[®]/Recormon[®]), DynepoTM control and usual negative control (NIBSC, National Institute for Biological Standards and Control). The arrows represent the DynepoTM injections. BI-scores are represented for each sample. Note that all D2 to D6 samples present scores > 0.84.

Application of classical WADA criteria

The application of the 3 classical WADA criteria [2] returned a very poor sensitivity (11%) regarding DynepoTM. Indeed, the 3rd criterion requires the second most intense basic band being at least twice more intense than the most intense endogenous one, what is not the case for most of DynepoTM-containing samples. Elimination of this criterion yielded a much better sensitivity.

References

- [1] F. Lasne, L. Martin, N. Crepin, J. de Ceauriz, *Anal Biochem.* 2002, 311, 119.
- [2] C. Ayotte, J.A. Pascual, G. Gmeiner, C. Reichel., F. Lasne, M. Saugy, *WADA technical document TD2009EPO*, 2008.
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- [4] P. Sottas, N. Robinson, M. Saugy, *The Athlete's Biological Passport and Indirect Markers of Blood Doping*, Doping in Sports, Springer, 2009.

IEF PROFILE CLASSIFICATION

Following bands assignment and GASepo integration, normalized volumes of bands 6 to -4 were selected as relevant features for classification. A partial least squares discriminant analysis (PLS-DA [3]) was performed with leave-one-subject-out cross-validation. A model with 3 latent variables (LVs) was computed with an explained variance R² was 0.68 and a goodness of prediction Q² of 0.63. The score plot indicated a partition between the positive and the negative sample classes, as well as the clustering of effort urines samples (figure 2A). The loadings revealed the contribution of each band to the class separation (figure 2B).

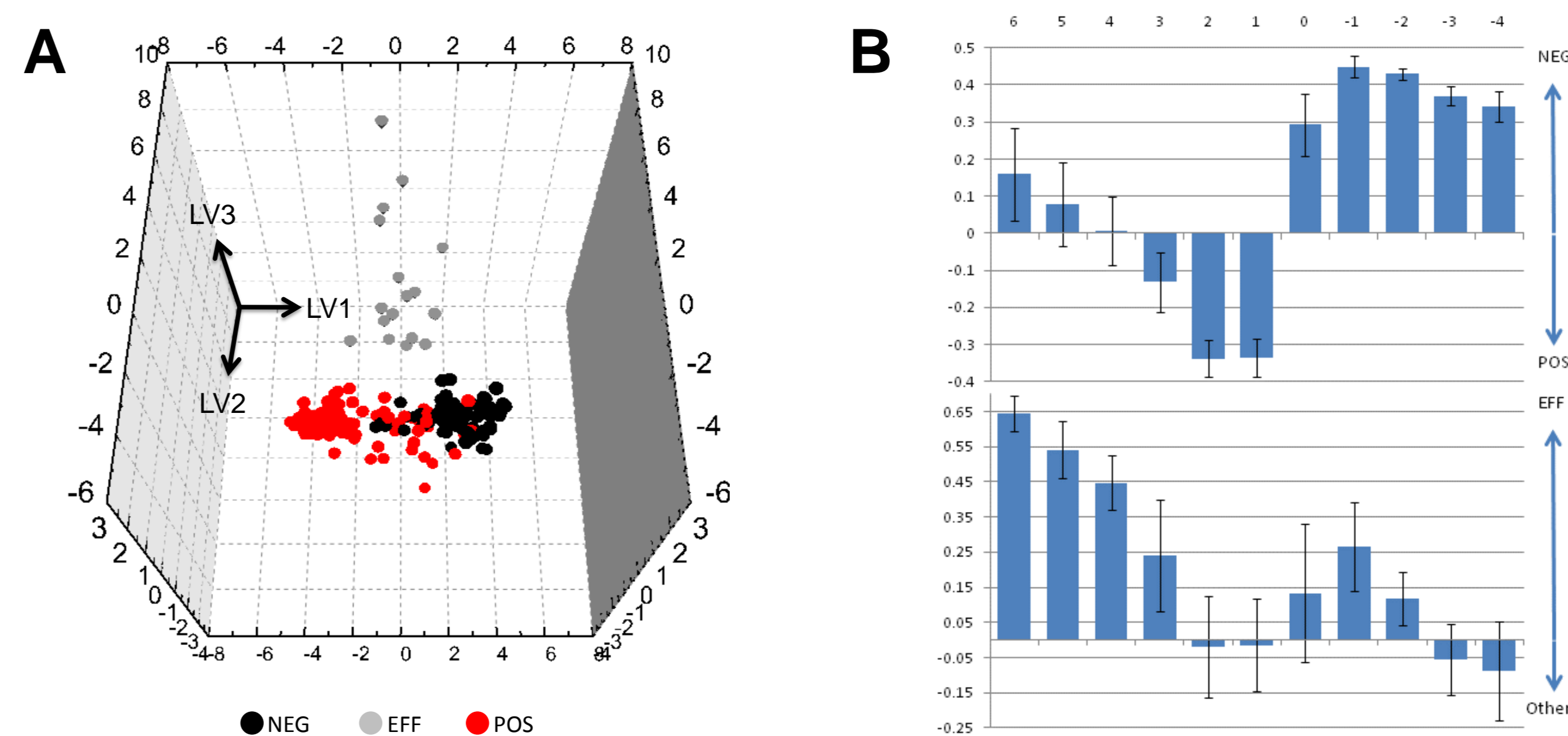


Figure 2A: 3D scatter plot depicting the distribution of the 2 classes of samples.

Figure 2B: Histograms representing the loadings of the 11 variables on LV1 and LV2.

A bands intensity score (BI-score) was computed for each sample, indicating how representative the sample is of the positive or negative class. The histogram of BI-scores obtained after cross-validation (figure 3A) and a Receiving Operating Characteristic (ROC) curve (figure 3B) were analyzed to determine a threshold value ensuring a perfect specificity, i.e. 0.84.

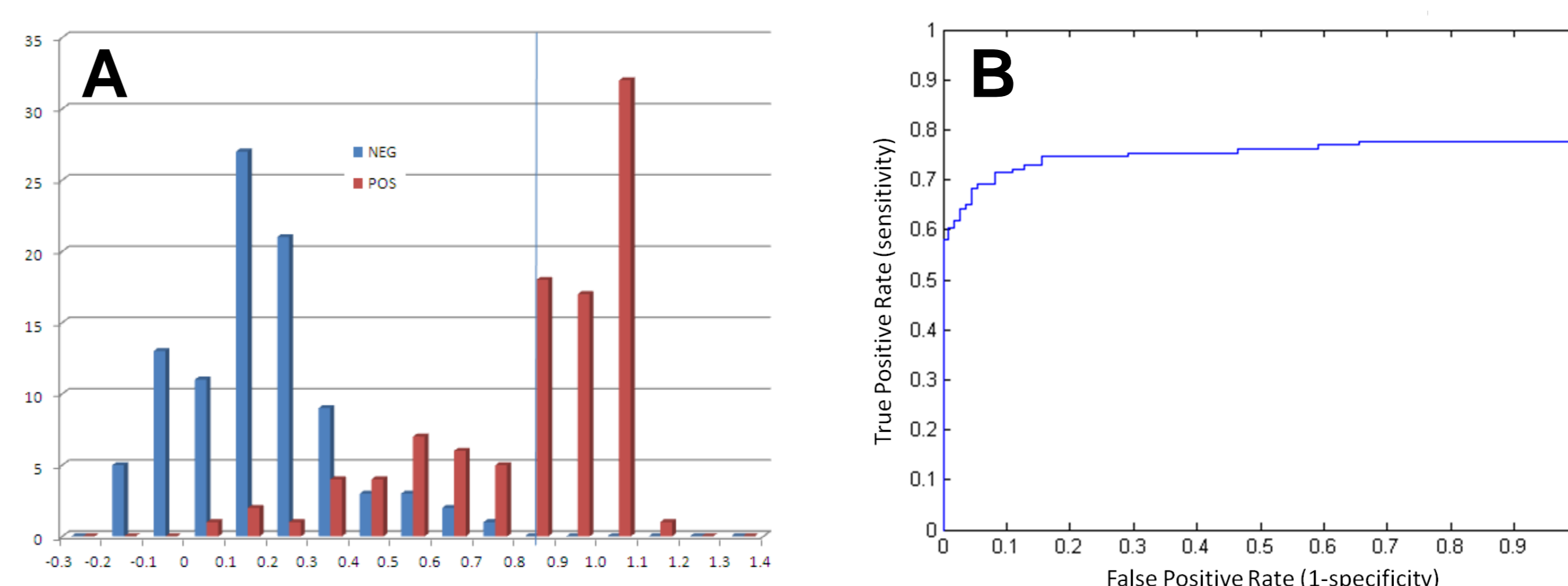


Figure 3A: Distribution of individual BI-scores obtained after cross-validation. The vertical line represents the cut-off (0.84) set up by the laboratory.

Figure 3B: ROC curve representing the true positive rate (sensitivity) versus the false positive rate (1-specificity) according to the cut-off limit.

DISCUSSION

Detection window

The detection window of DynepoTM in urine following multiple injections was evaluated from the set of 98 detectable samples collected during the excretion study. The detection window was defined as the period following the last DynepoTM injection during which more than 50% of the samples are classified as suspicious. On the basis of this definition, detection window of DynepoTM was evaluated as approximately 48 hours, what is fully concomitant with the detection windows established for epoetin- α and β regarding their respective half-lives.

Towards new criteria

Application of the pattern classification method suggests that a BI-score could be integrated in the procedure of EPO doping detection. Figure 4 proposes a decision tree for the identification of EPO doping. In case of a BI-score higher than the defined threshold, an expert assessment is required to ensure that all possible factors and issues are considered thoroughly.

For a BI-score lower than the threshold, there is still an opportunity to provide additional scientific evidence, such as a suspicious SDS-PAGE result and/or an atypical athlete's hematological passport [4].

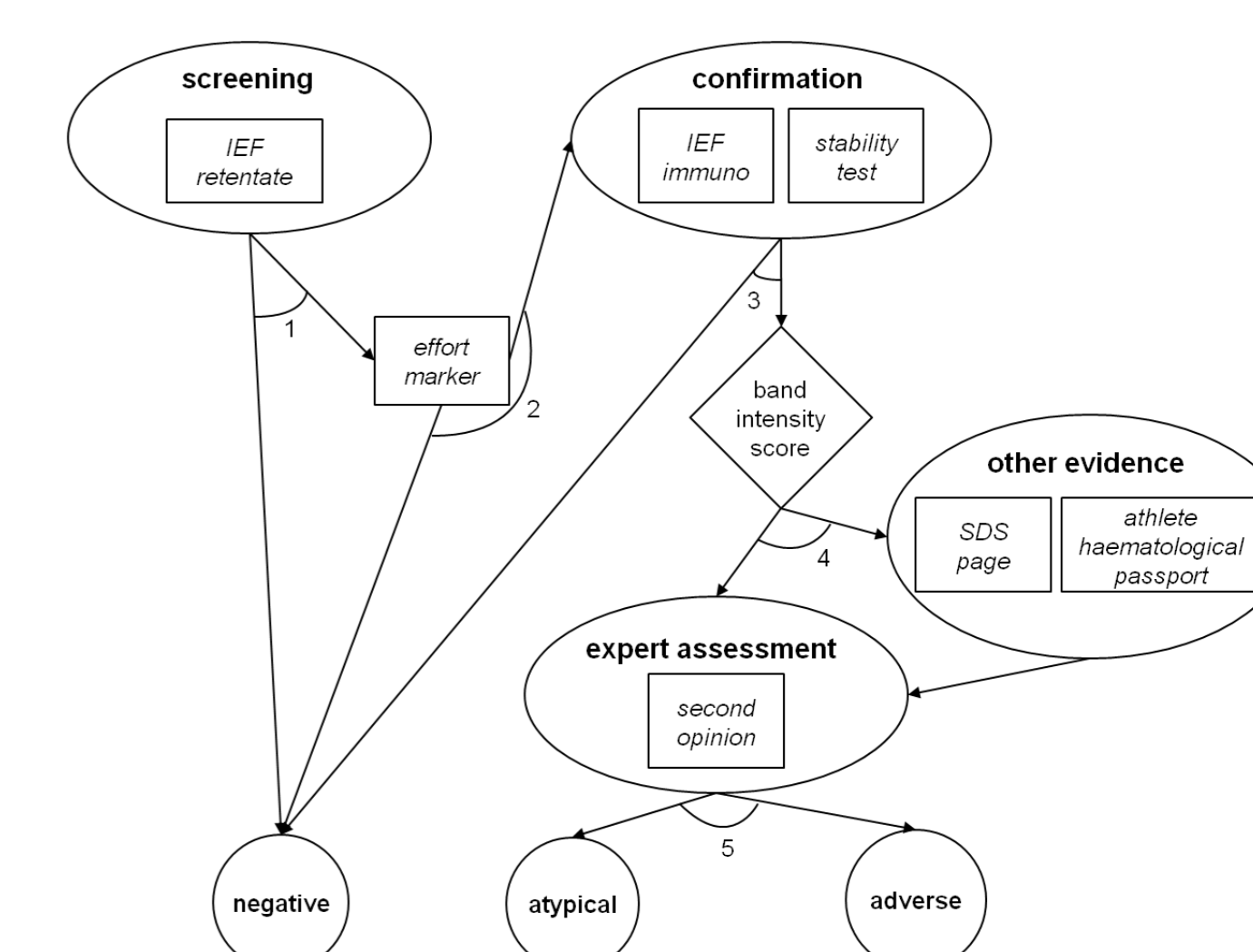


Figure 4: EPO doping decision tree