Project Review

"Purification of EPO in urine samples prior to detection by isoelectric focusing"

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Erythropoietin (EPO) is detected in urine by isoelectric focusing followed by immunoblotting procedures. The sample preparation involves mainly a concentration step by ultrafiltration on MW-selective filters. The retentate loaded on gel is therefore containing all other retained urinary proteins and the resulting impact is the frequent generation of distorted patterns of isoforms. It is suggested that a more selective purification step before loading the urine retentate on the IEF gel may improve the resolution of the isoform profiles, facilitate the interpretation and provide better quantification. Different chromatographic and affinity techniques of purification were evaluated in preliminary works. It was shown that chromatographic separation by anion-exchange chromatography improved significantly the resolution of the bands of urine retentates that would otherwise present smeared, diffused or arc-shaped bands. Immunoaffinity was also tested by purification through in-tube immunoprecipitation. This method allowed the total recovery of urinary EPO, and, combined with the elimination of other interfering proteins on gel, could also improve significantly the quality of the profiles.

It is suggested that adding sequentially both of these purification steps before loading the urine samples on IEF gels would increase the resolution and the clarity of the profiles of isoforms, and facilitate greatly the densitometric evaluation. This would contribute to standardize the interpretation of the results and limit its subjectivity. It can also perhaps represent an alternative to the second immunoblotting that is necessary to avoid non-specific binding of secondary antibody, with the dual advantage of increasing both the resolution and the sensitivity of the test.

In perspective, developing a low-scale purification method is the starting point to explore new approaches for the detection of urinary EPO such as analysis of biomarkers by mass spectrometry and 1D or 2D gel electrophoresis that were up to now, limited by the complexity of the urinary matrix.

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Results and Conclusions

The objectives of this study were to explore purification processes to improve the resolution and quality of the EPO isoform profiles on IEF gel, but also to set the basis for the exploration of new detection approaches by 1D gel electrophoresis or mass spectrometry, that were limited by the complexity of the urinary retentates. The resolution of urinary profiles of EPO isoforms was improved following purification, either by anion exchange chromatography or by immunoprecipitation of the retentates obtained after concentration of the specimens. The anion exchange resin approach was found to be of impossible routine application since rapid and irreversible deterioration of the column occurred. However, the immunopurification of urinary EPO isoforms was successfully developed and implemented in routine testing, which allows their analysis on SDS-PAGE gels.