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Validation and signal amplification of a flow cytometric method to detect homologous blood transfusion.

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Extended Abstract

Introduction

In endurance sport the maximum oxygen capacity is an important factor influencing athletic performance. An improvement of this factor is possible by increasing haemoglobin amounts with blood transfusion. Since 2004 homologous blood transfusion is commonly determined in routine doping control analysis using a procedure described by Nelson and Ashenden [1-5].
Due to the fact that there has no validation data been published for the use of IgM antibodies so far [3,4] and the fluorescence of the fluorophore FITC (Fluorescinisothiocyanate) is less intense compared to PE (Phycoerythrin) we aimed to improve the previously described method by using primary IgM antibodies in combination with secondary PE-conjugated antibodies.

Experimental

A panel of eight different primary antibodies and two different Phycoerythrin (PE) conjugated secondary antibodies were tested in this investigation. The flow-cytometer used in this study was the BD FACSArray instrument. Mixed Red Blood Cell populations were prepared from phenotype known donors. Linearity, specificity, recovery, precision, robustness (concentration of the primary antibody ± 15%) and interday-precision were controlled for every primary antibody which is used in the procedure. Linearity of the method was verified by the Mandel test [6] and was confirmed for every primary antibody.

Another objective of this project was to intensify the fluorescence of antigen expressing red blood cells with weak or heterozygous expression to improve and facilitate the interpretation of histograms.

A technique to amplify the fluorescence signal by two additional experimental steps was developed based on the use of a biotin-conjugated antibody directed against the fluorescent dye of the secondary antibody. In addition, streptavidin, conjugated to the same dye like the secondary antibody reacted with the biotin-conjugated antibody.

Results and Discussion

Validation:

The antibodies C, c, E, e showed higher detection and quantification limits, a higher coefficient of variation in intraday precision and lower recovery than the Duffy (Fya, Fyb) and Kidd (Jka, Jkb) antibodies(LOD: 1,1-2,0% compared to 0,3-0,5%) (LOQ: 3,7-6,8% compared to 1,3-1,8%) (Intraday Precision: 10,4-19,8% compared to 2,7-4,4%). The values for interday precision demonstrated that the method was robust for qualitative analysis but only antigens C and c provided adequate results for quantification. Furthermore, robustness regarding primary antibody concentration was proven for all antigens except for c and Jka. The method was able to clearly identify mixed red blood cell populations containing 0,3-2%.

Signal Amplification:

The technique of signal amplification resulted in a better separation of peaks. The modified blood sample treatment allows better interpretation of histograms in flow cytometry for homologous blood transfusion determination and minimises the risk of wrong negative samples.

References

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For details please refer to:

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