

## ***"Detection of autologous DEHP-free blood transfusion using a combination of multiple biomarkers in different matrices"***

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### **Project overview**

Autologous blood transfusions are a prohibited doping intervention in anti-doping field. The most promising attempt to detect autologous transfusions are the longitudinal measurement of different biomarkers such the Athlete Biological Passport (ABP).

Previously, the implementation of the detection of DEHP metabolite in urine showed to be an efficient method to detect autologous blood transfusion several hours after re-infusion. However, cheated athletes could easily use DEHP-free blood bags to prevent the detection of DEHP-metabolites in urine. For this reason, other biomarkers based on physiological response to transfusion of stored blood have to be discovered to detect autologous transfusion in anti-doping field.

In this project, we plan to perform clinical study in which volunteers will perform autologous DEHP-free blood transfusion. DEHP-free blood bags will be supplied thank to a laboratory-industry collaboration. Collection of different matrix such as plasma, serum and fingertip prick test samples will be carried out at different time points. In these samples multiple biomarkers such as blood cells, non- transferrin-bound iron (NTBI), total bilirubin, interleukin and circulating microRNAs will be measured and compared in order to detect autologous DEHP-free blood transfusion in recipient

### **Results and Conclusions:**

**BACKGROUND:** Autologous blood transfusion (ABT) is an efficient way to increase sport performance. It is also the most challenging doping method to detect. At present, individual follow-up of haematological variables via the athlete biological passport (ABP) is used to detect it. Quantification of a novel hepatic peptide called hepcidin and new urinary metabolites of plasticizers may be a alternative to detect ABT.

**STUDY DESIGN AND METHODS:** clinical randomized double-blinded two-phase study was conducted of healthy male volunteers who underwent ABT using DEHP-containing or BTHC blood bags. All subjects received a saline injection for the control phase and a blood donation followed by ABT 36 days later. The impact of ABT on hepcidin and plasticizers metabolites as well as haematological parameters, iron metabolism, and inflammation markers was investigated.

**RESULTS:** Blood transfusion had a particularly marked effect on hepcidin concentrations and long-term metabolites mono-(2-ethyl-5-carboxypentyl) phthalate and mono-(2-carboxymethylhexyl)phthalate compared to the other biomarkers, which included haematological variables. Hepcidin concentrations increased significantly: 12 hours and 1 day after blood re-infusion, these concentrations rose by 7- and 4-fold, respectively. No significant change was observed in the control phase. Surprisingly, considerable levels of urinary DEHP metabolites were observed up to 1 day after blood transfusion with BTHC blood bags. Levels of DEHP were high in BTHC bags (6.6%), the tubing in the transfusion kit (25.2%), and the leukocyte filter (22.3%).

**CONCLUSION:** Hepcidin quantification and urinary DEHP metabolite measurement is a cost-effective way to detect ABT in anti-doping field even when BTHC bags are used for blood storage.