***"Establishing doping-related reference distributions for cobalt in human urine and Reference values for cobalt in serum and urine after cobalamin (vitamin B12) administration"***

**Christian Reichel, Heinz Fröschl, Günter Gmeiner, Edi Benetka** (Seibersdorf Labor GmbH, Austria), **Damir Erceg** (Children's Hospital Srebrnjak, Zagreb, Croatia)

## Project Overview

The aim of the proposed project is the determination of reference distributions for cobalt in urine of elite athletes. Two cohorts will be compared: samples from athletes performing in endurance sports (i.e. sports prone to misuse of erythropoiesis stimulating agents), and samples from non-endurance sports. The reference distributions will help defining thresholds of cobalt in urine for doping control purposes, above which erythropoiesis was illegally stimulated by cobalt according to chapter S2.2 "Hypoxia-inducible factor (HIF) stabilizers" of the WADA Prohibited List 2016.[3]

In order to stimulate erythropoiesis, athletes have to apply $Co^{2+}$ ions (e.g. $CoCl_2$) at relatively high doses, which results in urinary concentrations, which are clearly distinguishable from cobalt in negative controls and after e.g. cobalamin administration. In total, ca. 600 urine samples (females/males equally distributed) will be analysed by ICP-MS as well as regarding their statistical properties (distribution type, outliers).

## Results and Conclusions:

The first part of the project ("Establishing doping-related reference distributions for cobalt in human urine") studied cobalt concentrations in urine samples of athletes. In total, 894 samples were collected worldwide and analysed by ICP-MS. About half of the athletes were from endurance sports (frequency of ESA-testing 30% or above according to WADA TD2014SSA), and about half from non-endurance sports (ESA-frequency < 30%). Furthermore, approx. 50% of the samples were from female, approx. 50% from male athletes. Non-parametric statistical analyses revealed for endurance-sports a median Co-concentration of 0.5 (0.8) µg/L (without and with specific gravity correction) for females (n=220) and 0.4 (0.6) µg/L for males (n=215). For non-endurance sports, the medians for females and males were 0.4 (0.6) µg/L (n=232) and 0.4 (0.4) µg/L (n=227), respectively. Based on the 5th and 95th percentiles the range of Co-values was 0.1 (0.2)-2.1 (2.4) µg/L for non-endurance and 0.1 (0.3)-4.9 (8.9) µg/L for endurance sports. A significant difference between the medians of Co measured in endurance and non-endurance samples (without or with SG correction) as well as the medians of Co in male and female samples (without or with SG correction) was found ($p < 0.01$). Seventeen samples (w/o specific gravity correction; all IC, 15 from endurance sports) showed Co-concentrations above 10 µg/L with a highest observed value of 948.0 (653.8) µg/L. Two of the 17 athletes declared cobalamin intake. Their values were

55.1 (78.7) and 10.5 (9.5) µg/L, respectively. In order to define possible decision limits (DL) at the 99.99% level, data were log-transformed and “outliers” removed to achieve normal distribution. Different DLs were obtained for males and females, as well as endurance and non-endurance sports and for data without and with SG correction.

The second part (“Reference values for cobalt in serum and urine after cobalamin /vitamin B12) administration”) investigated Co-concentrations in urine and serum samples after application of cobalamin (50/1000 µg cyanocobalamin oral, 1000 µg cyanocobalamin intramuscular, 1000 µg hydroxylcobalamin intramuscular). Only hydroxylcobalamin led to an increase in urinary and serum Co-concentrations. Maximum values were 11.7 (9.8) and 3.9 µg/L, respectively. No influence of cobalamin on ABP-blood parameters (HGB, Ret%) was found. However, the blood drawing system needs to be carefully selected and validated in order to not contaminate serum samples with cobalt as observed with the “butterfly needle system”.

In case a threshold for Co in athletes’ samples is defined in the future, it will be necessary to remove cobalamin in confirmation samples as recently shown by Knoop et al. (Rapid Commun Mass Spectrom. 2020;34(7):e8649).