Analytical attempts to monitor the application of testosterone doping - constituting as much as 66% of the all analytical findings for anabolic agents - focus mainly on the profiling of testosterone, its precursors and metabolites in urine samples, especially but not limited to the ratio of testosterone and epitestosterone. The establishment of reference ranges including a large variety of endogenous steroids shall contribute to a better detection of abnormal concentrations or ratios.

Urine as a sample matrix for investigating doping practices collects substances which have already passed the body, being like a waste bin their final destination and a remote collection matrix relating to the application of doping substances. Especially in the case of endogenous substances like steroids the influence of metabolism and excretion is levelling possible markers.

Leveling effects are expected to be smaller in serum samples. Especially in the case of parenteral application, normally having a lower amount of substance to enter the body due to the fact, that the liver passage represents no barrier and first pass metabolism, serum is expected to yield a better detection potential. Testosterone esters, the pharmaceutical form of testosterone applied within the frame of this project, besides unmodified testosterone, can be detected in serum samples, whereas they are undetectable in urine.

Due to the implementation of blood profiling as well as the detection of substances with minimal renal passage the access to blood and serum samples grows to get common practice and increasing importance in doping control. Steroid profiling is part of the concept of the athlete’s passport.

The aim of the proposed project is to target unique markers for parenteral application of testosterone preparations. To reach this goal three different parenteral routes of testosterone application are monitored. In addition a comparison to an oral application of a testosterone ester is done.
Results and Conclusions

The aim of the project was to target endogenous serum markers for application of testosterone preparations. In a clinical study, endogenous hormones in serum were monitored after the application of four different testosterone formulations; gel for transdermal delivery, transdermal patch, intramuscular injection, and oral tablets.

The analytes testosterone (T), epitestosterone (EpiT), androsterone (A), etiocholanolone (Etio), dihydrotestosterone (DHT), androstenedione (Adione), 5α-androstane-3α,17β-diol (5α-diol), 5β-androstane-3α,17β-diol (5β-diol), dehydroepiandrosterone (DHEA), 17-hydroxyprogesterone (17-OH-Prog), progesterone (Prog), luteinizing hormone (LH), follicle stimulating hormone (FSH) and sex-hormone-binding globulin (SHBG) were included in the project. The samples were assayed for LH, FSH, SHBG, and Prog using immunological methods. For all other analytes, a sensitive method was developed employing liquid-liquid extraction (LLE), derivatization, and liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS).

The study was open and randomized with no blinding procedure. It was divided into two periods during which each participant, randomly assigned, received an oral formulation in one of the periods, and a parenteral application (patch, gel or intramuscular injection) in the other period. All together 12 volunteers (men) participated in the study, and serum samples were collected for 4 weeks after drug application.

Changes in hormone serum concentrations and ratios were assessed using Wilcoxon matched pair test and Friedman ANOVA.

The serum concentrations did not significantly change for any of the hormones measured during the study. Furthermore, no significant changes were observed for the ratios T/LH, T/Prog, T/SHBG and DHT/T. A significant increase was, however, observed for the ratio T/17-OH-Prog in subjects treated with testosterone gel, compared to baseline values. In subjects treated with tablets, patch and intramuscular injections, a trend towards increased T/17-OH-Prog ratios was observed, but the increase was not statistically significant. The findings on elevated T/17-OH-Prog ratios are in accordance with previously published studies [1, 2].