

## ***“Detection of the long-acting testosterone formulation Nebido and influence on blood and steroid profile values”***

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### **Project overview:**

Although recent advances of steroid profiling have shown to substantially increase the detection sensitivity, still, the prevalent of misuse is larger than what doping control laboratories can detect. In particular the introduction of slow-release preparations such as T-gels and steroid patches poses anti-doping analysts for greater challenges as the metabolic footprint of misuse with these preparations is very small.

In 2009, Bayer introduced a long-acting T undecanoate depot Nebido® for therapeutic treatment of hypogonadism in males. Nebido® should be administered only once in two or three months, which is a much longer release period compared to other long-term T-formulations (e.g. Sustanon®). A dose of 1000mg T undecanoate should be injected in the muscle. It is claimed that the insufficient T-levels are restored and do not exceed normal serum levels and likewise cause less unwanted side-effects.

Also, it has been shown that such high T undecanoate doses can lead to elevated hematocrit levels. Moreover, sustained elevated T-levels can exert a beneficial anabolic effect on micro-damaged muscle tissue after long exercise to enhance recovery. Interesting features for endurance athletes, for whom the biological passport is a well-established tool to screen and record their doping test results. In this light, it is assumed that described effects can be noticed in both the steroidal and blood module of the biological passport and help to find Nebido® misuse in this category of athletes. This study aims to investigate the direct detectability of T-undecanoate as administered with Nebido® in blood as well as the influence on various blood and steroid parameters as recorded in the biological passport.

### **Results and Conclusions:**

Today the steroid passport is a very performant tool to detect abnormal variations in steroid profile markers. Although recent advances of current methods for endogenous steroids have shown to substantially increase the detection sensitivity, the prevalent of misuse is far larger than what doping control laboratories can detect. Particularly, slow-release preparations pose anti-doping analysts for great challenges as the metabolic footprint of misuse with these preparations can be very small. Nebido (Bayer) is one of such long-acting slow release T preparations, containing T undecanoate, that is used for therapeutic treatment of hypogonadism in males. In sports, slow releasing T formulation are applied to rapid repair of micro damage to muscles after exercise for faster recovery. In addition, it has been shown that such high T doses can lead to elevated haematocrit levels. Moreover, sustained elevated T-levels can exert a beneficial anabolic effect on micro-

damaged muscle tissue after long exercise to enhance recovery. Both features are interesting for endurance athletes, whereas T used to be related to cheating power athletes for its anabolic effects.

The T/E ratio was clearly altered by the use of Nebido as slow release T formulation and remain detectable using longitudinal following during 3 months. Androstenediol ratio and androsterone over etiocholanolone remain mostly unaltered, despite elevated concentrations. Minor steroid metabolites did not contribute to longer or better detection as likely the major pathways were not saturated. As a result the steroidomic model showed similar sensitivities as those observed for longitudinal evaluation of the T/E ratio.

A very sensitive method for the detection of T in saliva was developed and applied to the oral fluid samples collected after Nebido. Although, salivary T concentrations increased to 7 fold and detectable using population based reference ranges, the basal values were restored after 14 days. Oral fluid analysis of T did not provide any added value for doping control. Hence, the pharmacokinetics of salivary T are much different compared with the observations for T-gel, also a slow-release formulation.

Blood passports were created after the Nebido T supplementation and results demonstrated that the blood markers were significantly altered but did not results in atypical passport findings. The reticulocyte production was stimulated in the month after Nebido injection, after which a suppressive effect was observed in the second month. By this time haemoglobin was formed to give rise to an elevated plateau in the blood passport that remained below the upper individual limit. Also the off-score showed the alterations that was typical for bone marrow stimulation.