

## **“Detection of illicit administration of human chorionic gonadotropin using immunoaffinity extraction and mass spectrometric detection”**

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### **Aims**

The aim of this project was to provide a method for hCG detection based on immunoaffinity extraction and mass spectrometric detection. Furthermore, a small clinical trial was carried out in order to prove that hCG that has been administered to male athletes after illicit use of anabolic steroids, can in fact be determined in both serum and urine using immuno-MS methodology. Additionally a comparative study with the DELFIA immunoassay also was carried out.

### **Results and Conclusions**

The immuno-MS methodology has been optimized in order to enable the desired LOD and LLOQ, and has furthermore been validated according to existing guidelines. Using this method, a clinical study (approved by the Regional Committee for Medical Research Ethics) involving 24 healthy voluntary men was carried out. HCG was injected (either Ovitrelle or Pregnyl), and urine and serum samples were collected and analyzed. The method allowed detection and quantitation of administered hCG in urine, from day one until day ten after injection of hCG containing pharmaceutical. On day fourteen after injection, no trace of hCG can be detected. The method also allowed detection and quantitation of administered hCG in serum, from day one until day seven after injection of hCG containing pharmaceutical. On day fourteen after injection, no trace of hCG can be detected. Furthermore, the presence of different hCG variants in urine and serum samples has been demonstrated.

The immuno-MS method has been benchmarked against the DELFIA immunoassay and showed good correlation for the serum samples. For urine samples the DELFIA method showed significant lower hCG values than those obtained using the immuno-MS method. This is ascribed to the hCG instability in urine which has less influence on the immuno-MS method than on the DELFIA immunoassay. Both immuno-MS and DELFIA immunoassay detected hCG in equal long time after administration.

#### Conclusions:

The results demonstrate the methods capability of 1) detecting and 2) differentiating between the various hCG variants, thus demonstrating their presence in the biological matrix. Furthermore, the methodology can generate a 3) quantitative measurement of the hCG amount present in the patient as well as in healthy subjects to which hCG was administered. Based on this we conclude that the developed methodology of immunoextraction combined with mass spectrometric detection is capable of revealing the presence of illicit administered hCG in athletes. Additionally, the immuno-MS method gives similar results compared to the conventionally used DELFIA immunoassay.

### **Publications**

- H. Lund, K. Løvstetten, E. Paus, T.G. Halvorsen, L. Reubsaet. Immuno-MS Based Targeted Proteomics: Highly Specific, Sensitive, and Reproducible Human Chorionic Gonadotropin Determination for Clinical Diagnostics and Doping Analysis. *Analytical Chemistry* **2012**; Volume 84.(18) p. 7926-7932
- H. Lund, A.H. Snilsberg, E. Paus, T.G. Halvorsen, P. Hemmersbach, L. Reubsaet . Sports drug testing using immuno-MS: clinical study comprising administration of human chorionic gonadotropin to males. *Analytical and Bioanalytical Chemistry* **2013** p. 1569-1576
- H. Lund, A.H. Snilsberg, T.G. Halvorsen, P. Hemmersbach, L. Reubsaet. Comparison of newly developed immuno-MS method with existing DELFIA immunoassay for human chorionic gonadotropin determination in doping analysis. *Bioanalysis* **2013**; Volume 5 (5). p. 623-630