

## PROJECT REVIEW

### ***“Ultra-Sensitive Mass Spectrometric Detection of a rEPO Specific O-glycopeptide as an Unambiguous Proof of Doping—Follow-up (GOpep2)”***

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A project (acronym GOpep) was approved by WADA with the objective of detecting a Neu5Gc containing EPO O-glycopeptide using latest generation MS instruments (i.e. AB Sciex Qtrap 6500).

The EPO O-glycopeptide shows the lowest glycan heterogeneity, thus being the best choice to reach the necessary MS sensitivity. Results obtained showed that the glycopeptide isoform containing Neu5AcNeu5Gc was found to be the most abundant form with its triply charge species at m/z 810.3 giving the best signal. A limit of detection of around 2 IU EPO/L, from a standard preparation was achieved, compatible with the expected concentrations in human urine. An antibody against the peptide was also developed and initial results show that it also recognizes the glycopeptide. However, matrix effect when spiking real samples at very low concentrations, as well instrumental conditions to speed-up the analysis are still to be solved. The hypothesis of this project is that MS sensitivity has reached a status in which EPO glycopeptides, particularly O-glycopeptides as they present lower heterogeneity, are detectable in urine or blood samples. Using a proper combination of specific peptide immunopurification with other desalting techniques, matrix effects can be avoided and the required sensitivity for the EPO O-glycopeptide containing the non-human tag (Neu5Gc) reached.

Objectives:

- 1.- To improve sample clean-up by using the already developed polyclonal antibody against the peptide backbone and other desalting techniques.
- 2.-. To improve the nanoLC-MS/MS set-up using monolithic columns for high throughput and on-line sample clean-up.
- 3.- To develop monoclonal antibody for future use of the methodology if the use of the polyclonal proves successful.
- 4.- To validate the procedure in urine and serum samples