

## **Project Review**

### **"Effort urine: what is it?"**

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Endogenous and recombinant EPO isoforms have a different glycosylation pattern, and the resulting charge differences have been exploited to distinguish endogenous and recombinant isoforms by isoelectric focusing (IEF). Subsequently, the EPO isoforms can be visualized by a double immunoblotting technique. This 2-step procedure forms the basis of the actual urinary anti-doping test adopted by the World Anti-Doping Agency. In order to declare a urine sample positive to rHuEPO (recombinant erythropoietin), the isoelectric pattern has to fulfill certain requirements and notably the positivity criteria established in the Technical Document TD2004EPO. With time and experience, it appeared that some negative samples did not show the usual isoelectric pattern. Negative urine samples have a distribution (position and intensity) of the bands very alike the ones observed with NIBSC (Official negative urine control - National Institute for Biological Standard and Control) and for one reason or the other, some others do not have this distribution. It has been hypothesized that this phenomenon appeared specially when urine samples were collected after a strenuous physical effort.

The first step of this project is to mimic the efforts necessary to have a shift of the bands. The second step is to find and identify a possible urine marker which could help the scientist to interpret correctly the isoelectric profile. The third step is to eventually adapt the positivity criteria to strengthen interpretation of EPO profiles. The last step is to provide WADA with the necessary procedure in order to collect urine samples properly and avoid notably effort urine profiles. In order to have access to effort urines, we are planning to work in collaboration with the CMC (Centre Mondial du Cyclisme) and collect urine on a volunteer and anonymous basis. More than 10 top level athletes will be asked to perform repeated hard efforts (30-35 seconds, 500 m on a cycling track) over a period of 2 hours. Heart rates will be monitored as well as average and maximum power (SRM) to determine the kind of efforts necessary to obtain effort urines.