PROJECT REVIEW

"Stabilization of urine and blood in the doping control sealed containers after the addition by the kit manufacturers of stabilization agents"

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The aim of the study is the research for appropriate stabilisation agents to be added by the doping control kit container manufacturers during the manufacturing stage in the factory to the doping control kits in to order to suppress the active and microbial degradation process in urine and to protect the athlete's red blood cell (RBC) population and protein degradation in blood matrices. The degradation processes are the effects of the unavoidable problematic storage conditions during transportation of the samples from the collection cites to the Accredited Laboratories.

The present study focuses in the application of stabilization experiments to the urine samples in simulating sample transportation conditions for the following cases:

1. shifts to the erythropoietin bands profile in the confirmation of urine EPO analytical procedure
2. formation of 19-norandrosterone and 19-noretiocholanolone from androsterone and etiocholanolone in urine samples
3. microbial degradation which creates changes to the endogenous steroids urine profile
4. gonadotrophins degradation in urine samples
5. change to the RBC population profile in the blood transfusion method
6. protein degradation of growth hormone in serum samples

The stabilisation experiments will result in one or a mixture of stabilisation agents that could be added during the manufacturing stage of the doping control containers kit for urine, blood and serum. The urine or blood should dilute the stabilization agents and stabilize them for the transportation. Various aspects will be taken into consideration:

1. Types of degradation agents, like bacteria, that are responsible for the active conditions
2. Concentration of those stabilisation agents in urine and blood
3. Side effects from the presence of the stabilisation agents in the doping control kits, like creation of artefacts
4. Urine parameters like pH and sg

The sample transportation simulation conditions will be based on temperature and time parameters

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Results and Conclusions

Transportation of the doping control urine samples from the collection sites to the WADA Accredited Laboratories is performed almost everywhere at environmental temperatures. Those conditions, especially when the trasportation is conducted under high temperatures and delivery of the samples to the Laboratory is not immediate, microbial degradation of urine is a common phenomenon, which may result in misinterpretation of analytical data. Proteases discreetly introduced in urine specimen by athletes during the sampling procedure, to mask the administration of peptide hormones such as EPO is a practice that has already been reported.

WADA granted in 2005 the Accredited Doping Control Laboratory of Athens, Greece, to investigate various means for the stabilisation of urine against degradation. Even if chemical stabilisation was considered initially as the most probable mean, various physical stabilisation means were also investigated unsuccessfully. After the initial experiments and because not a single stabiliser can cover the full spectrum of the degradation agents, the composition of the stabilisation mixture included antibiotics and antimycotics for microorganisms and proteases inhibitors. Criteria for the inclusion of stabilisers in the mixture is to be effective, inexpensive and applicable to the sample collection plastic containers. The experiments performed in urine spiked with representatives of microorganisms of the normal microbial flora of urinary tract infections and environmental species and representatives of proteases of detergents and over-the-counter drugs with plant, animal and microbial origin. The experiments conducted in 37oC for 4 and 7 days to simulate transportation conditions favourite for the degradation of urine samples. The results proved that the stabilisation mixture substantially improves the quality of the urine samples against degradation.

Publications

1. M.TSIVOU, D.LIVADARA, D.G.GEORGAKOPOULOS, M.KOUPPARIS, J.ATTA¬POLITOU, C.G.GEORGAKOPOULOS, Stabilization of human urine doping control samples, Analytical Biochemistry, 388(2009)179.

2. M.TSIVOU, D.LIVADARA, D.G.GEORGAKOPOULOS, M.KOUPPARIS, J.ATTA¬POLITOU, C.G.GEORGAKOPOULOS, Stabilization of human urine doping control samples:

II. Microbial degradation of steroids, Analytical Biochemistry, 388(2009)146.

3. M.TSIVOU, H.DIMOPOULOU, I-P.LEONTIOU, D.G.GEORGAKOPOULOS, M.KOUPPARIS, J.ATTA-POLITOU, C.G.GEORGAKOPOULOS, Stabilization of human urine doping control samples: III. Recombinant Human Erythropoietin, Clinica Chimica Acta, 411(2010)448.

Presentations

1. M. Tsivou, D. Livadara, D.G. Georgakopoulos, M. Koupparis, J. Atta-Politou, C.G. Georgakopoulos: Preservation of urine doping control samples – Preliminary results,

Cologne Workshop 2008.

2. M. Tsivou, D. Livadara, D.G. Georgakopoulos, M. Koupparis, J. Atta-Politou, C.G. Georgakopoulos: Stabilization of endogenous steroids in sport urine samples, Cologne Workshop 2009.