

PROJECT REVIEW

“Characterization, diagnosis and prevention of various microbiological contaminants in urine samples”

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One of the aims of our ongoing study financed by WADA has been to find microbial contaminants of urine samples. We developed an in vitro simulation system for urine to mimic the storage and transportation conditions of urine prior to testing. Our hypothesis was that yeasts and bacteria are the most probable contaminants responsible for the adverse reactions in urine samples. Our preliminary results suggest that a complex microbial community such as that found in human saliva and feaces has a potential to modify the steroid profile of urine. The abovementioned findings have motivated us to accelerate the development in the area of microbial contamination and focus efforts on the real doping control samples. Here we are proposing an one year research project with following objectives

- (i) Identify contaminating microbes from real doping control samples (sent to Helsinki doping control site)
- (ii) Analyze the source of contamination for those samples
- (iii) Find the most important contamination parameters that affect the reliability of doping control analyzes
- (iv) Design routine laboratory test to find seriously contaminated doping control samples
- (v) Design procedures to eliminate microbial contamination or reduce its risk to the doping control analyzes

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Report and Conclusion

The microbial characterization of urine samples indicates that urogenital and gastrointestinal tract act as most probable contamination sources. All major microbial groups detected can be explained by non-intentional contamination sources. Intentional contamination is an existing possibility, but based on the results of this project we would put the research effort on the bacteria representing natural, unintentional contaminants.

Contaminated samples dominated by *Lactobacillaceae* and *Enterococcaceae* did not harbor as high microbial numbers as those dominated by *Enterobacteriaceae* and *Pseudomonadaceae*. Overall, the detected bacteria are known capable of altering the steroid profiles, emphasizing the importance of high hygiene level at sampling for a reliable doping control. However, in optimal conditions a low bacterial amount can increase exponentially to high levels in a short period of time.

Elevated pH is one of those indicators which are used in doping control laboratories to recognize microbial contamination. This screening parameter may be used together with a number of other criteria, e.g. the presence of free steroids in a urine sample, but has very limited selectivity alone. Abnormal smell or turbidity does not correlate with microbial growth.

Several studies have been carried out for the stabilization of human urine samples. None of the investigated physiological methods, including sterilization by filtration, ultraviolet radiation, or ultrasonication, have succeeded in preventing microbial growth. Chemical methods have been shown to be more efficient, but the introduction of any chemical substance into athletes' samples after collection may be difficult to approve legally. Consequently, rapid freezing has proved to be the only feasible method for stabilizing samples and preventing microbial activity.

Publications:

Ojanperä S, Leinonen A, Apajalahti J, Lauraeus M, Alaja S, Moisander T, Kettunen A. Characterization of microbial contaminants in urine. Drug Test. Anal. In press.

Presentations:

Alaja S, Apajalahti J, Leinonen A, Kettunen A, Ojanperä S, Kuuranne T, Lauraeus M. Characterization of microbial contaminants in urine. Poster in the Manfred Donike Workshop – 28th Cologne Workshop on Dope Analysis, 2010, Köln, Germany.