"*Proof of concept for the detection of clandestine compounds in urine samples using metabolomics*"

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PROJECT REVIEW

Detection of all performance enhancing drugs is essential to help maintain an environment in which drug free athletes can compete fairly. The modern athlete however is becoming more and more advanced in their efforts to avoid detection by anti-doping laboratories. This became evident in the BALCO conspiracy where compounds were made specifically to circumvent detection by laboratories for the athletes to gain an unfair competitive advantage. The current methods available to WADA laboratories are largely based on the detection of known compounds. A method which laboratories can use to detect new and previously unknown compounds - clandestine compounds is required. The project will apply advanced mass spectrometry and computing technology to develop a database for comparison to athlete samples. The comparison of an athlete's sample to the database will enable the detection of clandestine compounds such as new "designer" steroids. These clandestine compounds which are detected will undergo further analysis for identification purposes and thus will be able to be classified as either a prohibited substance or not. The developed techniques will be made available to all WADA laboratories.

Results and Conclusions

• The software package Expressionist from Genedata is capable of reliably detecting new compounds in a sample by comparing the peaks detected in the sample with all those in a library created from a set of blank samples. Of course if the new substance has the same exact mass (within ±0.005 amu) and lies within the retention time window (± 0.1 minutes) of a compound already in the library then it will not be detected.

• Optimisation of the parameters used for compound detection is a long and slow process owing to the multiple processes involved with each process typically having several adjustable parameters.

• Once optimised, the use of the software is relatively simple. Automatic processing of a single sample takes less than two minutes. Manual checking to determine if the non-annotated peaks detected are indeed not in typical blank samples may take another five minutes.

• The software is capable of processing the large data sets (hundreds of samples) needed to create blank libraries in less than a day.

• The sensitivity of new compound detection depends primarily on the signal strength obtained in the mass spectrometer. Detection levels down to 1 ng/mL have been achieved for some compounds.

• There is a trade-off between the ability to detect new compounds and the desire to have few if any false positives. The more peaks there are in the library the less likely it is that a new compound will be detected but fewer false positives from new blank samples will be produced. The best way of improving this would be if the retention time alignment could be improved to reduce the variability from ± 0.1 minutes to ± 0.05 minutes.

• The ideal of detecting all new compounds has not been achieved due to the desire of not having too many false positives in new blank samples. Having more than a few peaks to manually check in each sample will make use of the software impractical.

• It is unlikely that a laboratory would use the software to check all samples owing to the time involved. It is more likely that it would be used to check suspect or high risk samples. Once a new peak has been detected then further work using other techniques such as MSMS would be needed to assist in identification of the compound responsible for the peak.