

PROJECT REVIEW

"Identification and Characterization of Transcriptional Markers Diagnostic of Autologous Blood Doping"

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As autologous blood doping augments the athlete's blood with their own cells, detecting the procedure is challenging. Blood cell shape and distribution are being explored as potential markers for; however, changes in the transcript (the mRNA intermediate in the DNA-to-protein pathway) complement of the blood may be a more informative indicator of reinfused blood cells in an athlete. The transcript complement of a tissue is extremely sensitive to the environment, so blood cells that have been removed, processed and stored may show changes in transcript frequencies that could be diagnostic for autologous blood doping. RNA is an excellent candidate for doping tests as it is stable when stored correctly, quantifiable using commercially supported techniques, and measurable in very small blood samples

This proposal describes a one year pilot project using high-resolution transcription profiling of blood to identify transcripts with the potential to be used as a diagnostic test for autologous blood doping. The project has **three phases: 1)** identification of transcripts in whole and leukodepleted blood that represent genes that are activated or up-regulated in blood cells in response to post-withdrawal processing and/or short-term storage, **2)** use of simulated autologous blood doping to determine if these transcripts could be detected in a "doped" athlete; and **3)** characterization of the inter- and intra-individual variation of the transcripts shown to have potential test utility under a variety of conditions experienced by athletes (e.g. physical exertion, hypoxia/altitude, time of day, and time in menstrual cycle).

The ultimate goal of the project is to identify transcripts that are characteristic of stored blood, that are of sufficient quantity as to be identifiable in a recipient's circulation following reinfusion, and have patterns of expression that are consistent, predictable, and informative in athletes, which believe could form the basis for a blood-based, gene-expression test for autologous blood doping.

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Results and Conclusion

The aim of this study was to determine if there were genes overexpressed in blood in response to refrigerated storage that could be used as markers for the presence of stored blood in an athlete's circulation.

To be considered a potential transcript for follow-up, there had to be a minimum of a 10 fold expression difference (as this represented the typical dilution that would occur in an autologous reintroduction of a unit of blood into the 'doper's' circulation. We also felt that the overexpressed transcripts needed to be part of a unique pathway and not a variant of other commonly expressed pathways, as those would be unlikely sufficiently robust to serve as a diagnostic tool.

Outcome: Transcriptional analysis identified only a single non-hemoglobin gene that met the criteria for being a candidate for a test for blood storage. The gene was *GADD45G* (encoding: Growth arrest and DNA-damage-inducible protein GADD45 gamma). This gene is a stress response gene that has been shown to be up-regulated in circulating cells in response to 'behavioral stress' in animal models (e.g. restraint, Flint *et al.*, J Neuroimmunol. 2005; 167:34-44). This response would likely make it an unpromising candidate for a doping control assay as expression levels would likely fluctuate between athletes due to differential responses to perceived stress (e.g. doing control, imminent or recent competition).

The only other genes that fit the candidacy profile were 'rare' hemoglobins (delta and zeta). As the adult blood transcriptome is overwhelmingly made up of hemoglobin alpha and beta transcripts, it is unlikely that an assay sufficiently specific and sensitive could be developed based on differentiating between the highly homologous hemoglobin transcripts against this background.

Overall, the results of the project do not support the use of transcriptomic analysis of athletes' blood as a method for detecting the inclusion of stored blood (i.e. autologous blood doping).