PROJECT REVIEW

"GASEPO2- A Software Tool for Analysis of EPO Images after Isoelectric Focusing and Double Blotting"

G. Gmeiner, C. Reichel, I. Hollander, I. Bajla (ARC Seinersdorf Research, Seibersdorf, Austria), **C. Ayotte** (Doping Control Laboratory, Institute Armand Frappier, Montreal, Quebec, Canada), **D. Cowan** (Doping Control Laboratory, Kings College, London, UK), **C. Georgakopoulos** (Doping Control Laboratory, Athens, Greece), **P. Hemmersbach** (Doping Control Laboratory, Aker Hospital, Oslo, Norway), **R. Kazlauskas** (Australian Sports Drug Testing Laboratory, Pymble, Australia), **K. Muller** (Doping Control Laboratory, Kreisha, Germany), **J. Pascual** (IMIM, Barcelona, Spain), **W. Schänzer** (Doping Control Laboratory, German Sports University, Cologne, Germany)

Since the development of an analytical method for the direct detection of the application of recombinant erythropoietin in urine samples by Lasne et. al. (Nature, 2000, 405, 635), great efforts have been made to implement the method in different laboratories and to standardize and harmonize the interpretation of the data obtained by isoelectric focussing, double blotting and chemiluminiscence detection. Up to now no final decision has been made by the labs accredited for the cited method, which sets of criteria for positivity are to be applied for the interpretation of the gels. The use of different software products with different tools of data interpretation interferes the search for a common basis of gel interpretation.

The aim of this project proposal is to develop a reliable and easy-to-use software package called GASEPO2, specially designed for quantification of recombinant erythropoietin in urine samples as a standardized and unified tool for the use across doping-control laboratories worldwide thus providing for international harmonization of EPO analysis. This software is an advancement of the precursor software GASEPO1, designed as research prototype in 2003.

The development includes research in design of decision criteria based on image information as well as in mathematical methods of image analysis including image segmentation and 3D visualisation. By using the concept of band segmentation for the differentiation between band and background, the isolated single band serves as basis for further calculation rather than a 1 D transformation of the entire lane. GASEPO2 will equally well handle images taken by a dedicated digital camera for chemiluminescence or scanned from a photographic film. The software will come with a validation package that allows auditing. The implementation of alternative evaluation methods in the software will contribute to and facilitate the process of finding and harmonizing the decision criteria.

Results and Conclusions

"GASEPO-2: A software tool for analysis of EPO images after isoelectric focusing and double blotting"

The Project fulfilled the original goals, namely:

• A software tool has been created that has been generally accepted by the doping control community and is now being routinely used in 75% of all laboratories doing epo-analytics.

• Thus, the product of the Project contributed directly to the superordinate aim of standardization and harmonization of the epo analytics.

• An effective epo-analytics expert network has been created.

• Scientific know-how has been extended and disseminated via a number of scientific publications.

• WADA-funded research in doping control has been promoted by a number of project-related presentations for broader public.

Publication Activity

The following publications have been released with direct connection to the GASepo Project:

- I.Holländer, I.Bajla, M.Minichmayr, G.Gmeiner, Ch.Reichel. **GASEPO -System for Analysis of Images Generated in EPO Doping-Control.** *In: J.Jan, J.Kozumplík,I.Provazník, editors, Analysis of Biomedical Signals and Images. Proceedings of the 17th International EURASIP Conference BIOSIGNAL 2004, Brno, Czech Republic, June, 2004. Pages 273-277.*

- I.Bajla, I.Holländer, G.Gmeiner, Ch.Reichel. **Analysis of Epo Images after Isoelectric Focusing and Double Blotting.** *In: Proceedings of the Second Intern.Conference Biomedical Engineering, Innsbruck, IASTED, 2004, 16-18 February 2004. Pages 228-233.*

- H.Ramoser, J.Biber, I.Bajla, I.Holländer. **Segmentation of Electrophoretic Images in Doping Control.** *In: Proceedings of the Intern. Conference on Mathematicsand Engineering Techniques in Medicine and Biological Sciences, METMBS'*04, 2004, Las Vegas, USA, 15-16 June, CSREA Press. Pages 467-470.

- I.Bajla, I.Holländer, D.Heiss, R.Granec, M.Minichmayr. **Object Classification in Images for Epo Doping Control Based on Fuzzy Decision Trees.** *Applications of Neural Networks and Machine Learning in Image Processing IX, part of the IS&T/SPIE Symposium on Electronic Imaging 2005, San Jose, USA; 16-20 January, 2005.*

- I.Holländer, Ch.Reichel, G.Gmeiner. **EPO-Analytik: Unsichtbares sichtbar** gemacht. *Labor Praxis, Dezember 2004.*

- I.Holländer, I.Bajla, M.Minichmayr, G.Gmeiner, Ch.Reichel. **GASepo: system for Analysis of images Generated in EPO Doping-Control Proteomics.** 2nd *Int.Symposium of the Austrian Proteomics Platform, Seefeld in Tirol, Austria, 24-27 January, 2005.*

- I.Bajla, I.Holländer, G.Gmeiner, Ch.Reichel. **Quantitative Analysis of Images in Erythropoietin Doping Control.** *Medical and Biological Engineering and Computing, 43, (2005) pages . Pages 403-409.*

- D.Heiss-Czedik, I.Bajla. Using Self-Organizing Maps for Object Classification in Epo Image Analysis. *Measurement Science Revue. Vol. 5, Section 2, 2005.*

- S. Štolc, I.Bajla. Improvement of Object Segmentation and Classification via Correction of Epo Image Distortion. *Proceedings of the 5th Int. Conf.* Measurement 2005, Smolenice, Slovak Republic, May 15-19, 2005, IMS SAS Bratislava, (eds): I.Frollo, M.Tysler, and V.Juras. Pages 212-215.

- I.Holländer, I.Bajla, G.Gmeiner, Ch.Reichel, M.Minichmayr. **epoCAM: A system for chemiluminescence image acquisition in doping control.** *Proceedings of the 5th Int. Conf. Measurement 2005, Smolenice, Slovak Republic, May 15-19, 2005, IMS SAS Bratislava, (eds): I.Frollo, M.Tysler, and V.Juras. Pages 190-193.*

- S. Štolc, H.Penz, K.Mayer, D.Heiss. **Verfahren zur Ermittlung von Helligkeitswerten (Method for Intensity Values Determination).** *Austrian Patent Application A833/2005.*

- I.Holländer, I.Bajla, M.Minichmayr, G.Gmeiner, Ch.Reichel. **GASepo: System for** analysis of images generated in EPO doping-control proteomics. *Abstract and poster in Manfred Donike Workshop (23rd Cologne Workshop on Dope Analysis).*

- I.Bajla, I.Holländer, M.Minichmayr, G.Gmeiner, Ch.Reichel. **GASepo – a Software Solution for Quantitative Analysis of Digital Images in Epo Doping Control.** *Computer Methods and Programs in Biomedicine (2005), Vol. 80. Pages 246-270.*

- S.Štolc, I.Bajla. Improvement of Band Segmentation in Epo Images via Column Shift Transformation with Cost Functions. *Medical and Biological Engineering and Computing, Vol.* 44, (2006). Pages 257-274.

- S. Štolc, I.Bajla. Improvement of Band Classification in GASepo Systems Used in Epo Doping Control. In: Proc. of the 18th Biennal Int. EURASIP Conference Biosignal, June 27-29, 2006, Brno, Czech Republic, VUTIUM Press. Pages 281-283.

- I.Bajla, I.Holländer. Verfahren zur Analyse von Bandenbildern - Dopingkontrolle (Method for band image analysis). Austrian Patent 500 963.

- Ch.Reichel, I.Holländer, G.Gmeiner. **Improvement in the Background Correction of EPO Images.** Abstract in Manfred Donike Workshop (Cologne Workshop on Dope Analysis, Cologne 2006).

- I.Holländer, G.Gmeiner, Ch.Reichel, I.Bajla, M.Minichmayr. **GASepo -The Three Years of a Successful WADA Project.** *Poster at the Int. Conference Say No to Doping, Bucharest, Romania, November 8-11, 2006.*