



**ICAW 2015
1-4 SEPTEMBER
ANTWERP, BELGIUM**

CONFERENCE GUIDE

11TH INTERNATIONAL COMET ASSAY WORKSHOP

ANTWERP UNIVERSITY, BELGIUM

P-13: The use of comet assay to assess global DNA methylation in human biomonitoring studies.

Carla Costa^{1,2}, Ana Catarina Alves³, Solange Costa^{1,2}, Amadeu M.V.M. Soares³, Marta S. Monteiro³, Susana Loureiro³ and João Paulo Teixeira^{1,2}

¹Portuguese National Institute of Health, Portugal

²EPIUnit - Institute of Public Health, University of Porto, Portugal

³Department of Biology & CESAM, University of Aveiro, Portugal

The Comet assay is a valuable tool for the detection of DNA damage in genotoxicity and human biomonitoring studies. Throughout the years, this biomarker has undergone several adaptations in their protocol in order to increase its sensitivity and the possible outcomes. By including an additional step of DNA digestion with lesion-specific endonucleases, the comet assay can provide information regarding the type of DNA damage detected in cells. The use of these enzymes has also allowed the development of a methylation-sensitive modified version of the comet assay. This version enables the routine measurement of global methylation, as well as CpG island DNA methylation in a variety of cells while simultaneously determining the genetic integrity of examined cells (Wentzel, 2012). Briefly, it makes use of isochizomeric restriction enzymes HpaII and MspI (that display differential sensitivity to DNA methylation) to characterize methylation outside CpG islands and restriction enzyme NotI to determine DNA methylation in CpG islands. The technique has been recently adapted to a medium-throughput version (Lewies, 2014) that allows the simultaneous analysis of a larger number of samples and overcomes some technical problems. Nevertheless, this technique has not yet been carried out in human biomonitoring studies. In this context, the aim of this work was to make use of this version of the comet assay to characterize global DNA methylation in approximately 50 human samples. Samples were analysed by the methylation-sensitive modified version of the comet assay (medium-throughput) and by ELISA based assay. Data obtained with both methods were compared and reproducibility of the methylation-sensitive modified version of the comet assay determined. Results obtained contribute to knowledge on the feasibility of this version of the comet assay and its possible usage in human biomonitoring studies as an epigenetic biomarker.

Acknowledgements

This work was supported by The Portuguese Science Foundation (FCT) through CESAM (UID/AMB/50017/2013) and CNRS/INEE - National Center for Scientific Research/Institute of Ecology and Environment, via OHMI - International Observatory Hommes-Millieux.

Carla Costa and Marta S. Monteiro are supported by the grants SFRH/BPD/96196/2013 and SFRH/BPD/45911/2008, respectively, funded by FCT (QREN - POPH - Type 4.1 - Advanced training, subsidized by the European Social Fund and national funds of MEC).

References

Johannes F. Wentzel and Pieter J. Pretorius (2012). Investigating the Role DNA Methylations Plays in Developing Hepatocellular Carcinoma Associated with Tyrosinemia

Type 1 Using the Comet Assay, DNA Methylation - From Genomics to Technology, Dr. Tatiana Tatarinova (Ed.), ISBN: 978-953-51-0320-2, InTech, doi: 10.5772/34802.

Lewies, A., Van Dyk, E., Wentzel, J. F., & Pretorius, P. J. (2014). Using a medium-throughput comet assay to evaluate the global DNA methylation status of single cells. *Frontiers in Genetics*, 5, 215. doi:10.3389/fgene.2014.00215