

Effect of storage on the suitability of whole blood samples to analysis by Comet assay

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DNA oxidation occurs in many diseases, *e.g.* cancer, cardiovascular disease, inflammatory disease, neurodegenerative disease, where it may have a primary pathological role and/or be a consequence of the disease [1]. The measurement of DNA damage in peripheral blood cells (PBC) may serve as a marker of systemic oxidative stress, or act as a surrogate for the assessment of oxidative stress in diseased tissues.

The European Standards Committee on Oxidative DNA Damage (ESCODD) identified the potential for artefactual damage formation during sample storage and workup [2]. This has meant that blood samples, for example, require rapid isolation of PBC, by density centrifugation, prior to storage in an appropriate cryopreservative. This is time-consuming and labour intensive, and is unlikely to have been performed for many of the existing biobank collections, requiring new, prospective collections. Combined, this has been a significant hurdle to the use of blood samples in large human biomonitoring studies. An assay is required that is relatively simple to perform, and is applicable to, ideally, small volumes of whole blood which have been frozen rapidly. ESCODD identified Comet assay as an assay which possesses a low risk of artefact formation and requires small numbers of cells.

We have evaluated the suitability of alkaline comet assay to the analysis of DNA damage in PBC, without prior isolation from whole blood. We have examined various technical issues related to comet slide preparation and the effect of storage conditions. Promising early results suggest that the comet assay is amenable to the analysis of DNA damage in PBC, without isolation from whole blood, even following long-term storage at -80 °C. This has led to the development of a working protocol for the facile collection and storage of blood samples using 'pin-prick' samples, with negligible artefactual formation of damage and the preservation of pre-existing damage.

[1] Evans, MD., Dizdaroglu, M. and Cooke, MS. (2004) Oxidative DNA damage and disease: induction, repair and significance. *Mutat. Res. Rev.* **567**, 1-61.

[2] ESCODD (2002) Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus. *Carcinogenesis*, **23**, 2129-2133.