



**Karolinska  
Institutet**

**Institutionen för biovetenskaper och näringsslära**

## **International validation of the comet assay and a human intervention study**

**AKADEMISK AVHANDLING**

som för avläggande av medicine doktorsexamen vid Karolinska  
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av

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## ABSTRACT

The comet assay is an established, sensitive method extensively used in biomonitoring studies. The methods' advantages include; **a)** only a small cell sample is required, **b)** possibility to measure damage in practically any cell type, **c)** ability to measure heterogeneity in response within a cell population, **d)** relatively fast and economical procedure, and **e)** various applications of the method, which allow measurement of a range of different DNA lesions as well as DNA repair.

Several guidelines for the comet assay have been published, but no standardised protocol exists as yet. There are considerable differences between the protocols used by different research groups, which negatively affect inter-laboratory comparisons of results. Several experts in the field have highlighted the need for multi-laboratory, international validation studies, to assess intra- and inter-laboratory reproducibility and to investigate sources of variability in the results.

The papers in this thesis can be divided into two parts; one part that deals with international inter-laboratory validation studies and methodological aspects of the comet assay (paper I-III), and the other part covers a human intervention study with antioxidant capsules consisting of many different antioxidants in low doses for which the comet assay has been applied (paper IV-V).

The inter-laboratory validation trials in papers I-II indicate that the participants can detect dose-responses of both DNA breaks and oxidatively damaged DNA in coded cells, but that there is a large inter-laboratory variation in the reported values. This variation can in part be explained by differences in comet assay protocols and in image analysis. The inter-laboratory variation was decreased, but not completely removed, by calibration with ionising radiation.

In paper III we verified that several protocol steps significantly affected the outcome of the comet assay, including **a)** density of the agarose gel, **b)** extent of enzymatic incubation, **c)** duration of alkaline treatment, and **d)** time of electrophoresis, as well as the strength of the electric field applied.

In a parallel placebo-controlled, double-blind intervention study, overweight middle-aged men were supplemented for six weeks with a multivitamin supplement consisting of a range of antioxidants in doses resembling those achieved by a healthy diet (paper IV-V). In spite of elevated levels of seven out of eight measured antioxidants in the blood, the intervention did not affect the level of oxidation of lipids or DNA. Many intervention studies with good design report similar null findings. It is preferred to consume antioxidants through a healthy diet, and dietary supplements are not recommended for cancer prevention.