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Use of the CometChip® for the *in vitro, in vivo* and Fpg-modifed assay

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The high throughput 96 macrowell system with a spatially encoded array of microwells patterned in agarose, has gained in popularity in recent years. The commercial version (CometChip®) has only been used when applying the standard *in vitro* version of the assay, and comparisons with the more commonly used protocols, such as the 2 gels/slide, have not been published. In this work, new protocols to allow the adoption of the enzyme-modified comet assay as well as the analysis of *in vivo* samples were developed. Results obtained using the CometChip® were compared with those obtained with the classic 2 gels/slide version.

TK6 cells were treated with different concentrations of methyl methanesulfonate (MMS) or hydrogen peroxide (H2O2), for the standard version of the assay, or potassium bromate (KBrO3) in the case of the enzyme-modified version. Appropriate solvents were used as negative controls. For the *in vivo* comet assay, snap frozen samples of liver, kidney and duodenum were obtained from Wistar rats orally dosed with 200 mg/kg MMS and sacrificed after 3 hours.

Adapting CometChip® protocol for the use of the enzyme formamidopyrimidine DNA glycosylase (Fpg) requires modifications in washing steps before the enzyme incubation, together with changes in overlay agarose and enzyme concentrations. In the case of the analysis of *in vivo* samples, adjustments in the temperature of certain steps were needed. After adapting the CometChip® protocol, results obtained in the in vitro standard and Fpg-modified comet assay and in the *in vivo* assay are comparable to the ones obtained when using the 2 gels/slide protocol. It is important to note that different systems need different protocols to obtain comparable results.

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