

Flash-comet and CometChip, a comparison with the standard comet assay

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The alkaline comet assay is a widely used genotoxicity technique. However, its throughput has always been an aspect for improvement and some modifications have been made in that direction, such as developing the CometChip® (Bio-Techne) (1), based on a 96 well-format, or reducing the duration of alkaline unwinding and increasing the voltage gradient using a lithium-based solution, the so-called Flash-comet assay (2). The objective of this work was to compare the results obtained with these modifications with a standard version of the assay.

TK6 cells were treated with methyl methanesulfonate (MMS) and hydrogen peroxide (H₂O₂) at different concentrations. In the standard version of the assay, 2 gels/slide format was used; cells were lysed for 1 h followed by 20 or 40 min of alkaline treatment and electrophoresed at 1.2 V/cm during 20 min. Concerning the Flash-Comet, cells were lysed for 1 h at pH 8.5 or 10, followed by 2.5 min of alkaline treatment in a lithium hydroxide solution (pH 12.5) and an electrophoresis of 1 min at 5 V/cm in the same solution. The CometChip® was used following the manufacturer's protocol. Briefly, cells were loaded in the chip, covered with agarose, subjected to an alkaline treatment of 40 min and electrophoresed at 1 V/cm for 50 min. In this case, the Fpg-modified version was also performed in KBrO₃-treated cells.

The sensitivity of the Flash-comet was lower compared to the standard version of the assay, being unable to detect the MMS-induced damage. Dose response curves, with slight differences, were obtained with both compounds using the CometChip® and standard version. Concerning the Fpg-modified version, almost no DNA in tail (%) was obtained using the CometChip®.

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1. Ge, J. et al (2014). CometChip: a high-throughput 96-well platform for measuring DNA damage in microarrayed human cells. *Journal of visualized experiments: JoVE*, (92), e50607.
2. Bivehed, E. et al (2020). Flash-comet: Significantly improved speed and sensitivity of the comet assay through the introduction of lithium-based solutions and a more gentle lysis. *Mutation research. Genetic toxicology and environmental mutagenesis*, 858-860, 503240.