

Featured Publication Note

Dr. Westerink and colleagues

Toxicology and Drug Disposition, MSD, Oss, The Netherlands

Development and Validation of a High Content Screening In Vitro Micronucleus Assay

Determination of genotoxic potential is an important factor in drug discovery. Drug compounds with genotoxic side effects give rise to the formation of micronuclei (MN). In this study, researchers have developed and optimized a high content screening (HCS) in vitro MN assay (IVMN) with CHO-K1 and HepG2 cell lines. Scoring of MN in regulatory IVMN assays is often labor-intensive and performed manually, resulting in inter-/intra-observer variations. Here, the MN scoring algorithm from the Acapella® High Content Imaging and Analysis Software was used.

Imaging was performed using the Operetta® High Content Imaging System and Harmony® High Content Imaging and Analysis Software, followed by analysis using Acapella software. An overview of the image analysis strategy used is shown in Figure 1. Binuclear cells were first identified by the detection and pairing of nuclei. The cytoplasm of the cells was then detected, followed by the MN.

Cells were treated with various genotoxic reference agents and the fraction of binuclear cells with MN was assessed using Acapella software. The concentration-dependent induction of the fraction of binuclear cells with MN after exposure to five model compounds is shown in Figure 2.

The classification of genotoxic compounds as either clastogens or aneugens is an important consideration when developing MN assays. Standard methods for classification are laborious, since additional staining, imaging, and analysis is often needed. Here, researchers incorporated a method to discriminate between aneugens and clastogens into the image analysis strategy, based on size-classification of the identified MN.

The HCS image analysis-assisted IVMN assay developed here was able to efficiently detect genotoxic potential and allow differentiation of genotoxins into clastogens and aneugens. This may prove to be a useful strategy to assess genotoxic potential in the early stages of drug discovery.

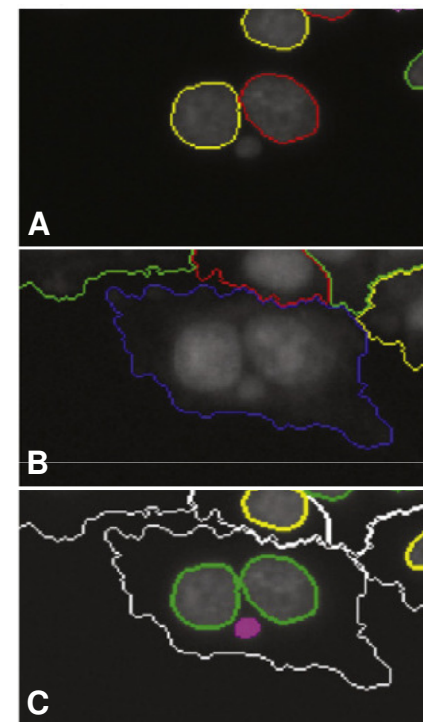


Figure 1 (above): Representative images for CHO-K1 cells, showing the steps involved in image analysis. The three main steps during image analysis are shown: A) Detection and pairing of nuclei, B) Detection of cytoplasm, C) Detection of micronuclei within the cytoplasm of a bi-nucleate cell (depicted as a pink dot).

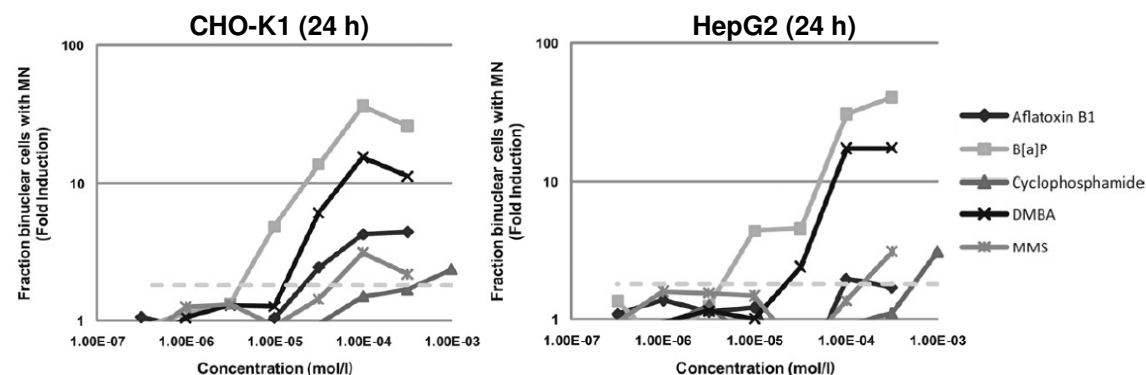


Figure 2 (left): HCS in vitro micronucleus assay. To determine the effectiveness of the newly developed assays, CHO-K1 and HepG2 cells were treated with five model compounds; Aflatoxin-B1, benzo[a]pyrene (B[a]P), cyclophosphamide, 7,12-dimethylbenzanthracene (DMBA) and methyl methanesulfonate (MMS). The dose-response curves for each compound are shown. The dashed line indicates the genotoxicity threshold. All compounds showed a genotoxic effect after 24 h.