Evaluation of an Automated Scoring System (Cellomics) for the In Vitro Micronucleus Assay in CHO-K1 Cells

Andrew Scott[1], Sophie Malcomber[1], Sharon Maskell[1], Claire Moore[1], Sam Windebank[1], Dolores Diaz[2] and Paul Carmichael[1]

[1]Safety and Environmental Assurance Centre, Unilever, Sharnbrook, Bedfordshire, MK44 1LQ, UK.

[2]Cerep Inc., 15318 NE 95th Street, Redmond, Washington 98052, USA.

Introduction

The *in vitro* micronucleus (IVM) assay is a standard genotoxicity assay that is used to assess the potential for compounds to induce chromosome damage in cultured mammalian cells. As with other tests for chromosome damage, the IVM assay uses gram quantities of compound and significant amounts of personnel and time resources. Therefore, the application of automated technologies to this assay is desirable. Traditionally, micronucleus detection in the IVM assay is undertaken by manual scoring methods. Automation of this phase of the assay has the potential to reduce the time required to generate data. The Cellomics Micronucleus bioapplication software allows for the automated and rapid quantification of binucleated cells and micronuclei required for the assay. Additionally, the Cellomics-based IVM assay is performed using 96-well plate format and as such has dramatically reduced compound requirements (milligram quantities of material).

The aim of this study was to evaluate the performance of the Cellomics-based IVM assay compared to results generated using manual scoring methods for a diverse set of 30 compounds.

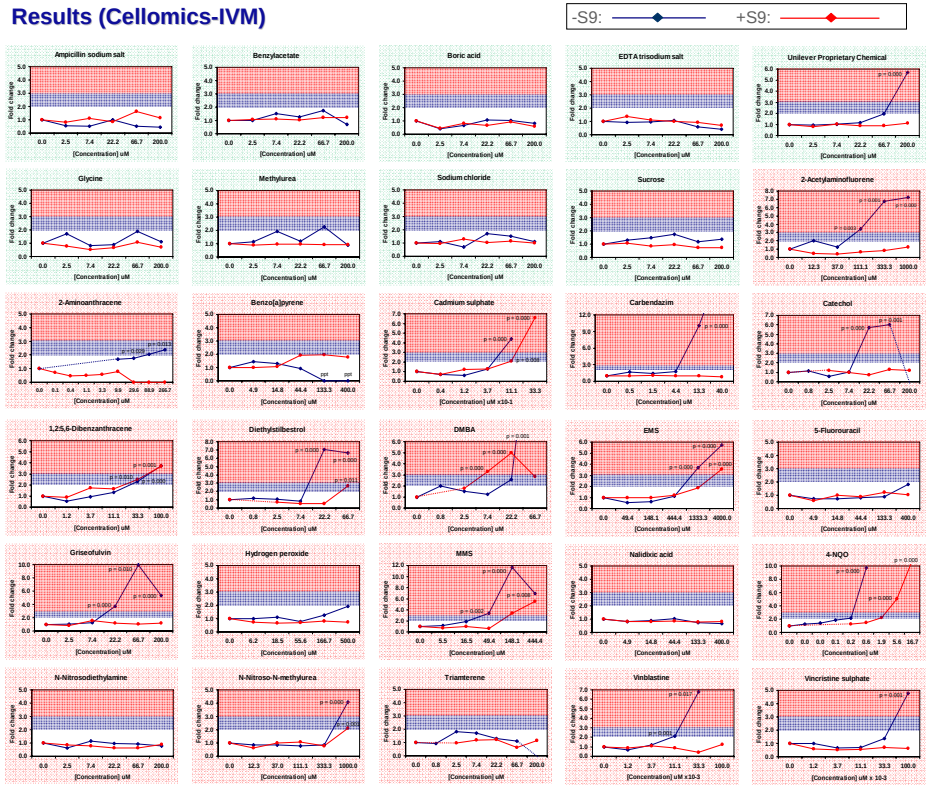
Materials and Methods

CHO-K1 cells were seeded into collagen coated 96-well plates and incubated overnight at 37°C. Cells were stained with a dye to allow the boundary of the cytoplasm to be identified and then treated with test compounds for 3 hours (+S9) or 24 hours (-S9) to evaluate micronuclei formation. Test compounds were assessed in duplicate at 10 concentrations separated by 3-fold dilution intervals. After removal of the test compound the cells were treated with Cytochalasin B (for 27.5 hours). A permeability dye was then added (for the identification membrane permeability changes associated with apoptotic and necrotic cells), following which the cells were fixed and stained with a DNA/nuclear dye (for the identification of micronuclei and the assessment of the total nuclear count). The plates were then scanned using an Arrayscan HCS reader and the Micronucleus Bioapplication (Cellomics).

The percentage of micronucleated binucleate cells is determined. A marginally-positive result is defined a value significantly higher than controls (t-test, $p<0.05$), and at least 2-fold higher than controls. A positive result is defined as a value significantly higher than controls (t-test, $p<0.05$) and at least 3-fold higher than controls.

All compounds were coded by Unilever before being sent to Cerep for genotoxicity assessment.

Results (Cellomics-IVM)

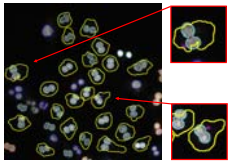


Summary of results

Compound ID	Study Call	
	Cellomics	Manual
Ampicilin sodium salt	NEG	NEG
Benzylacetate	NEG	NEG
Boric acid	NEG	NEG
EDTA trisodium salt	NEG	NEG
Unilever proprietary chemical	POS	NEG
Glycine	NEG	NEG
Methylurea	NEG	NEG
Sodium chloride	NEG	NEG
Sucrose	NEG	NEG
2-Acetylaminofluorene	POS	POS
2-Aminoanthracene	NEG	POS
Benzo[a]pyrene	INC	POS
Cadmium sulphate	POS	INC
Carbendazim	POS	POS
Catechol	POS	POS
Cyclophosphamide (positive control)	POS	POS
1,2:5,6-Dibenzanthracene	POS	POS
Diethylstilbestrol	POS	POS
9,10-Dimethyl-1,2-benzanthracene	POS	POS
Ethyl methanesulphonate	POS	POS
5-Fluorouracil	INC	POS
Griseofulvin	POS	POS
Hydrogen peroxide	INC	POS
Methyl methanesulphonate	POS	POS
Mitomycin C (positive control)	POS	POS
Nalidixic acid	NEG	POS
4-Nitroquinoline N-oxide	POS	POS
N-Nitrosodiethylamine	NEG	POS
N-Nitroso-N-methylurea	POS	POS
Triamterene	NEG	POS
Vinblastine	POS	POS
Vincristine sulphate	POS	POS

Manual data based on published findings

POS: Positive; NEG: Negative; INC: Inconclusive



Results and Conclusions

We have conducted an evaluation study at Cerep Inc. with a range of 21 mutagenic and 9 non-mutagenic compounds with varying modes of action (results determined from studies in which standard micronucleus scoring methods were used). All compounds were coded by Unilever before being sent to Cerep for genotoxicity assessment. Overall, the concordance of the Cellomics-based IVM assay with the IVM assay using manual scoring methods was 83.3% (specificity was 88.9% and sensitivity 80.1%). These data demonstrate that the Cellomics micronucleus bioapplication has the potential to automate the in vitro micronucleus test whilst reducing compound requirements to milligram quantities. Efforts to optimise the S9 reaction are in-progress to potentially improve the concordance of the Cellomics-IVM with manual scoring methods.