Ultra High Speed Automated In-Vitro Micronucleus Analysis

Geert Kalusche, Lynne Smith, Elizabeth P Roquemore and Nick Thomas*  
GE Healthcare Limited, Amersham Place, Little Chalfont, Buckinghamshire, England, UK HP7 9NA. Tel: +44 (0)1295 2052 6439; Fax: +44 (0)1295 2052 6230; e-mail: nick.thomas@ge.com

Introduction

Micronucleus induction is a key characteristic of genotoxic compounds. Analysis of micronucleus formation resulting from DNA strand breakage (clastogenic) or interference with chromatin segregation (aneugenic) is an important component of toxicity screening of new drug candidates. Manual scoring of micronuclei assays is time consuming and subject to operator variance, bias and error. Automated analysis of micronuclei assays allows very significantly faster analysis and consistently objective scoring. We have developed software for the In Cell Analyser 3000 for analysis of micronucleus assays using standard Cytoselect®-8 black protocols. The software segregates mono-nucleate and bi-nucleate cells and outputs micronuclei frequency and proliferation index with an image analysis time of ~2 minutes for a complete 96 well plate. The software is also compatible with analysis of micronuclei assays imaged using In Cell Analyser 1000 via image conversion to In Cell Analyser 3000 format.

Methods

Micronuclei assays were carried out in CHO-K1 cells grown in imaging grade 96 well plates in clear black, Greiner or Viewplate, Poland. Cells were exposed to solvent or test compounds for 24 hours, Cytoselect®-8 13µg/mL was added and cells incubated for a further 24 hours. Following fixation in ethanol for 30 minutes at room temperature, total cellular protein was stained with FITC 0.1µg/mL cells washed in PBS and nuclear and micronuclear DNA stained with Hoechst 13µg/mL. Plates were imaged on In Cell Analyser 3000 and In Cell Analyser 1000 D0x objective using Fluorescein and Hoecht excitation and emission filters (Fig. 1).

Analysis

In Cell Analyser 3000 images and In Cell Analyser 1000 images converted using the In Cell 3000 converter were analyzed using automated image analysis software (Fig. 2). Analysis parameters were set to identify and quantify micronuclei corresponding to published guidelines [1,2]: i.e. micronuclei were located within the cell cytoplasm, detached from nuclei with similar staining intensity and 0.33 nuclear diameter.

Results

Exposure of CHO-K1 cells to increasing concentrations of the clastogens Mitomycin C and Bleomycin and the aneugen Etoposide and Diethylstilbestrol resulted in dose-response dependent induction of micronuclei (Fig. 4). For each compound micronucleus frequency (µ% bi-nucleate cells with micronuclei) increased to a maximal value followed by a decrease in micronuclei frequency accompanying a drop in the proliferation index, measured as the ratio of bi-nucleate to mono-nucleate cells, indicative of compound toxicity (Fig. 5). Regression analysis of data (Fig. 6) excluding data points from wells with frank toxicity was used to derive EC50 values for test compounds (Table 1).

The accuracy of the automated analysis procedure was validated against manual counting of micronuclei using replicate wells exposed to Mitomycin C (Fig. 7).

Table 1. EC50 values for micronuclei induction

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC50 ( µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitomycin C</td>
<td>2.9 x 10^-8</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>4.1 x 10^-8</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>6.9 x 10^-8</td>
</tr>
<tr>
<td>Etoposide</td>
<td>1.8 x 10^-5</td>
</tr>
</tbody>
</table>

To perform the analysis (Fig. 3) cell nuclei were identified by thresholding the nuclear channel (blue) followed by segmentation of mono-nucleate and bi-nucleate cells based on nuclear intensity (DNA content) and nuclear form factor (symmetry). Thresholding of the cytoplasmic channel (green) combined with operator defined search boundaries based on segmented nuclei was then used to define a search perimeter for micronuclei meeting defined size and intensity criteria.

Discussion

In this work we have described the development and evaluation of software for automated analysis of micronucleus assays. Analysis of micronuclei occurring in CHO-K1 cells grown in 96 well plates was performed in two platforms using In Cell Analyser 3000 and converted images from In Cell Analyser 1000. Analysis was performed in the Cytoselect®-8 black protocol with fluorescence detection of Hoechst nuclear dye and micronuclear content. Automated analysis is faster than manual analysis and consistently objective.

Conclusions

- We have developed a very high speed automated analysis procedure for measurement of in-vitro micronucleus induction providing a flexible and powerful analysis solution for high throughput screening of compounds for genotoxicity
- The software is compatible with standard micronucleus in-vitro assay protocols and provides options for analysis of assays performed in the presence or absence of a cytokinesis block
- The software provides the option to use a third channel permeability marker to reject dead cells from micronucleus analysis
- Analysis and data output is completed in <2 minutes for an entire 96 well plate

References