



Abstracts

Oral Communication

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Genotoxicity Assessment using Yeast

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We have developed a simple assay for assessment of the genotoxic potential of chemical compounds. The assay detects the activation of DNA repair using a green fluorescent protein (GFP) reporter system in budding yeast. Thus the assay measures genotoxic activity rather than relying on a mutation end point. The objective of this study was to establish how effectively the yeast assay anticipates the results of the regulatory tests for genotoxicity, i.e. The Ames test, in vitro Mouse Lymphoma assay and in vitro and in vivo Micronucleus tests.

A typical experiment started with 500 ml of compound at 0.5 mM in 4% DMSO. Aliquots of test compound were serially diluted in 96 well microplates to give ten 75- μ l samples. A 75 μ l aliquot of yeast cells in medium was added to each sample and the plates then incubated overnight at 25 °C. The 2-fold dilution steps and yeast addition were carried out either manually or using a commercially available liquid handling robot. Cell density and fluorescence measurements were used to provide quantitative measures of cytotoxicity (reduction in cell proliferation, or cell cycle progress) and genotoxicity (fluorescence normalized to cell yield) and these results were checked against internal control compounds included on the assay plate. Subsequent automated data handling generated a cytotoxicity result (positive, or weak positive, with lowest effective concentration, or negative) and a genotoxicity result (positive, or weak positive, with lowest effective concentration, or negative).

Previously, we reported a limited validation exercise of the assay (Afanassiev et al., 2000). Here we report data for 87 compounds. For each compound, the genotoxicity result was scored against corresponding results for Ames, in vitro Mouse Lymphoma and in vitro and in vivo Micronucleus tests (results compiled from the literature). Thirty eight compounds were positive in the yeast test. Two of these were considered “false positive” on the basis that all other test data was negative. These were 3-amino-1,2,4-triazole (Ames and ML negative) and tritolyl phosphate (Ames and ML negative). Forty nine compounds were negative in the yeast test. Two of these were considered “false negative” on the basis that all other test data was positive. These were cumene hydroperoxide (Ames positive) and ICR191 acridine mutagen (Ames and ML positive).

This study reveals that the yeast test gives a very low incidence of 'False Positive' results. Regulatory genotoxicity tests are expensive and time consuming. There has been a lot of interest in the development of cheaper, faster screens to give an early preview of the Ames test. The yeast test is simple to perform and may be used to give a prediction of regulatory test results.

Reference

Afanassiev, V., Sefton, M., Anantachaiyong, T., Barker, G., Walmsley, R., Wöfl, S., 2000. *Mutat. Res.* 464, 297-308.

Keywords: Yeast; GFP; DNA repair; Genotoxicity; Cytotoxicity; Screening

Poster Presentation

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Inter-Laboratory Validation of the GreenScreen Assay

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The GreenScreen Assay (Yeast Genotoxicity Screen) has been developed to provide a rapid genotoxicity assessment for pharmacologically interesting chemicals. The objective of this study was to establish the reproducibility and reliability of this yeast (*Saccharomyces cerevisiae*) DNA damage reporter system. The inter-laboratory validation was conducted to a quality standard based on GLP.

Yeast is now more commonly being used in the detection and evaluation of carcinogens. It is an attractive model system because it is an eukaryote. It is a useful model for the testing of highly bactericidal compounds such as antibiotics, which preclude the use of the regulatory or screening Ames Test. The yeast has been genetically engineered to produce a fluorescent protein in proportion to the activation of their DNA repair systems. The harder its DNA repair systems work the brighter the glow. The yeast cells, serial dilution of test article solution, genotoxic control (methyl methanesulfonate), cytotoxic control (methanol) and diluent (2% dimethylsulphoxide) were manually mixed in 96-well microplates, then incubated overnight at 25 °C.

Cell density and fluorescence measurements (through incorporation of reporter gene RAD54) were used to provide quantitative measures of cytotoxicity (proportional to cell proliferation) and genotoxicity (proportional to fluorescence normalized to cell yield). The test system included automatic data capture, which generated interpretations (tabular and graphical) for cytotoxicity and genotoxicity. The assessment levels for each are: positive cytotoxicity = all dilutions produce cell densities that are $\geq 20\%$ lower than blank; weak cytotoxicity \leq three consecutive dilutions produce cell densities $\geq 20\%$ lower than blank; negative cytotoxicity = no dilution produces cell densities $\geq 20\%$ lower than blank and positive genotoxicity = all dilutions produce brightness values $\geq 30\%$ higher than blank; weak genotoxicity \leq three consecutive dilutions produce brightness values $\geq 30\%$ higher than blank; negative genotoxicity = no dilution produces brightness values $\geq 30\%$ higher than blank.

The validation tested a variety of compounds and correlated the data to that produced by the Ames Test and in vivo studies (compared with extensive literature base and in-house data). Of the 13 compounds tested at the validation site, five were tested by the developers of the system and eight by personnel validating the system, to determine any possible inter-laboratory and inter-personnel variation. Methyl methanesulfonate and 1-methyl-3-nitro-1-nitrosoguanidine were confirmed as genotoxins; Ampicillin and Caffeine were confirmed as non-genotoxins; Urethane and N-nitroso-N-methyl urea were confirmed as Ames false negatives and 8-hydroxyquinoline was confirmed as an Ames false positive. The remaining compounds, Cisplatin, Phenol, Sulfamethoxazole, Bleomycin sulphate, Benzoyl chloride and Hydroxyurea required re-tests at different dose ranges, due to variable data, to confirm or otherwise the results initially gained.

The test was performed with rapid end-point determination and concurrently measured cytotoxicity and genotoxicity. The data generated by the developers and validators at the test site were in most cases in agreement with previous in-house data (confirming the lack of inter-laboratory and inter-personnel variation) and support the use of the GreenScreen Assay as a useful and rapid genotoxicity assessment tool.

Keywords: Yeast; Screen; Fluorescent protein; Cytotoxicity and genotoxicity
