Predictive screening tools for genetic toxicology assessment in drug discovery

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Background

Early identification of genotoxicity is critical in drug discovery. The GLP-standard OECD 487 *in* vitro micronucleus test (MNT) is a cornerstone of regulatory genetic toxicology assessment and is used for assessing chromosome damage *in-vitro* and can detect chromosome breakage (clastogenicity) and chromosome loss (aneugenicity). A positive result in an *in-vitro* micronucleus test can be problematic for a compounds development at a point where significant time and cost have already been invested.

To address this challenge, Scantox offers miniaturised non-GLP screening assays based on their MNT GLP-counterpart: 96-well MNT and MicroFlow® assays. These assays are well suited to early hit-to-lead and lead optimisation phases of drug discovery, requiring <50 mg of compound, providing higher throughput and rapid data turnaround.

Here we assess the concordance of the OECD Flask MNT with the miniaturised 96-well MNT format and also the MicroFlow® assay for micronucleus detection, testing recommended genotoxic (clastogenic and aneugenic) compounds with and without metabolic activation.

Materials & Methods

TK6 lymphoblastoid cells were utilised for two treatment schedules: 3 hours in the presence of a rat liver S9 based metabolic activation system and continuous treatment, 24 hours in the absence of S9 mix.

A stock of each test item was formulated in DMSO and serially diluted to formulate the required test item concentrations. The highest starting concentration for each test item was decided based on ICH S2, OECD Guideline 487 (2023) or on previous studies found in the literature. TK6 cultures were treated at 1% v/v. Concentrations of the negative and positive controls were equivalent to that in test item cultures.

For Flask and Plate Micronucleus Test: Cultures were counted and Relative Population Doubling (RPD) and cytotoxicity calculated. This was then used to select concentrations for analysis according to OECD 487 Guideline. Cell densities were adjusted and slides prepared using cytology centrifuge. Following fixation, slides were stained using Acridine Orange in DPBS. The number of mononucleate cells with and without identifiable MN were recorded. The number of MN per concentration were compared with the solvent control.

For MicroFlow assay: Following compound incubation, treated cultures were stained with two nucleic acid according to the MicroFlow® protocol to identify nuclei/micronuclei (SYTOX Green Dye) and dead/dying cells (EMA dye) and analysed via flow cytometry. An established gating strategy allowed for identification of micronuclei and measurement of cytotoxicity.

		Flask MNT	96 Well MNT	MicroFlow®
Cell Density		$1.5x10^5$	$2x10^5$	$2x10^5$
Dilution Interval		1.5	1.25	1.25
Number of Concentrations		16	24	16
S9 Percentage		1%	2%	1%
Positive Controls	3H+S9	CPA 4 µg/mL	CPA 2.5 + 4 µg/mL	CPA 5 µg/mL
	Continuous	COL 7.5 + MMC 30 ng/mL	COL 7.5 + MMC 30 ng/mL	MMC 60 ng/mL

Table 1: Key components of each micronucleus test methodology

Results

	Flask MNT		Plate MNT		MicroFlow®	
Compound	3H+S9	Continuous	3H+S9	Continuous	3H+S9	Continuous
Cyclophosphamide	POS	POS	POS	POS	POS	NEG
Mitomycin C	POS	POS	POS	POS	POS	POS
Colchicine	POS	POS	POS	POS	POS	POS
Benzo[a]pyrene	POS	NEG	POS	NEG	POS	NEG
4-Nitroquinoline 1-oxide	POS	POS	POS	POS	POS	POS
Eugenol	NEG	POS	NEG	POS	NEG	POS
Vinblastine	POS	POS	POS	POS	POS	POS

Table 2: Outcome of micronucleus test for all test items in each treatment and format. POS= positive, NEG= negative. For Flask and Plate MNT, the outcome was deemed positive and negative based on if the following parameters were met (3/3 = POS, 0/3 = NEG): 1) One or more concentrations produced MN number greater than 33 (based on in-house historical control database). 2) One or more concentrations produced a statistically significant increase in MN (based on Fishers-Exact One-Sided statistical test P=<0.05). 3) Statistically significant result in a trend test (Cochran-Armitage P=<0.05). For MicroFlow®, the outcome was deemed positive if %MN fold change >3-fold above vehicle baseline and at that dose, if %EMA positive fold-change is <4-fold above vehicle baseline.

Results

Figure 1: Comparison of cytotoxicity curves and % fold change of micronuclei versus vehicle in each treatment arm across flask and plate micronucleus test formats and Microflow® assay for four of the test items. CPA = Cyclophosphamide, MMC = Mitomycin C, COL = Colchicine, VIN = Vinblastine Sulfate.
There was some variation in the cytotoxicity curves as well as the % fold change of micronuclei, however the overall outcome matched across all test item treatments with the exception of CPA Continuous –S9. In the flask and plate MNT continuous –S9 studies, CPA generated a weak positive result, contrasting with the negative result generated in the continuous MicroFlow study, though the cytotoxicity and % fold change MN were comparable across assay types. This difference in classification is attributed to the differences in criteria for classifying a positive result, where MNT assays utilize an approach based on historical control data, and the MicroFlow® assay utilizes a fold-change cut-off.

Conclusion

Based on the overall outcomes of the 7 compounds tested, the two formats of the micronucleus test, flask and 96 well, and the MicroFlow® assay were considered to be concordant. This data demonstrates the use of 96-well format of the micronucleus assay and the MicroFlow ® assay as highly predictive tools, ideal for use in early drug discovery.