The Ames MPF™ Assays:
Novel Mutagenicity Testing in Liquid Microplate Format using *S. typhimurium* TA98, TA100, TA1535 and TA1537

Sini Flückiger-Isler and Markus Kamber
Xenometrix, Gewerbestr. 25, CH - 4123 Allschwil, Switzerland

Introduction:

Genetic toxicity testing has moved towards the earlier stages of drug discovery in order to identify genotoxic liabilities of new compounds in the pipeline. Because in early development many compounds are available in very small quantities, a liquid microplate version of the original Ames plate incorporation test with different S. typhimurium tester strains was developed to decrease compound consumption and to increase the through-put of the assay.

The complete Salmonella plate incorporation test includes at least 2 frameshift strains, usually TA98 and TA1537 or TA97, and at least two base-pair strains. TA100 is generally the most sensitive of all tester strains, but some mutagens are positive in TA1535 only. TA98 is already successfully used in the Ames II microplate assay, in combination with TAMix, a mixture of strains to detect specific base-pair mutations. Recently we were able to manage the high spontaneous reversion rate of TA100 such that it could be used instead of TAMix in the Ames MPF™ 98/100 without loss of sensitivity. TA1537 or TA1535 were until now not available in the microplate format.

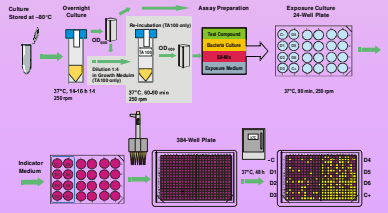
The mutagenic response to 24 reference compounds was examined with TA98, TA100, TA1537 and TA1535 in the microplate format, and the results were compared with published plate incorporation data. Depending on the strain tested and on the citation chosen, concordance between the two assay formats was 89 - 100%. Certain mutagens exclusively reverted TA1537 or TA1535, but not TA98 or TA100, irrespective of the assay format.

These new Ames MPF™ tests by Xenometrix using a liquid format and 384-well microplates offer a time and cost-effective pre-regulatory alternative to the plate incorporation method. As both formats use the same *Salmonella* strains, results can be compared with existing data sets. The new test kits include ready-to-use media and quality-controlled bacteria and allow rapid screening of a large number of compounds. They consume six times less test substances and consumables than the plate incorporation method, and reduce hands-on time.

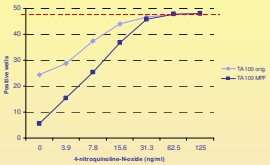
Test method:

The Ames MPF™ assays are performed in 384-well plates with the histidine auxotroph *Salmonella typhimurium* tester strains TA98 and TA1537 (frameshift mutations) and TA100 and TA1535 (base-pair substitutions). After overnight growth, exposure with test chemicals is performed in 24-well plates (6 concentrations in triplicate, together with solvent and positive controls) in the absence and presence of S9 mix. After treatment, a specially formulated medium containing a pH indicator and lacking histidine is added. Each well of the 24-well plate is aliquoted into 48 wells of a 384 well-plate and incubated for two days to allow revertant bacteria to form colonies. Mutagenicity (bacterial growth) is measured colorimetrically by a color change (pH drop) from purple to yellow.

The data presented in this poster were done with 4 concentrations.



Results:



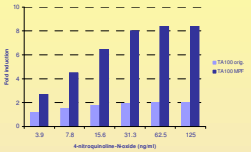


Figure 1: Comparison of the original TA100 strain with TA100 MPF.

Due to its greatly reduced spontaneous reversion rate the „Fold Induction over Zero Dose" is significantly higher and therefore suitable for the microplate format with a upper limit of 48 wells

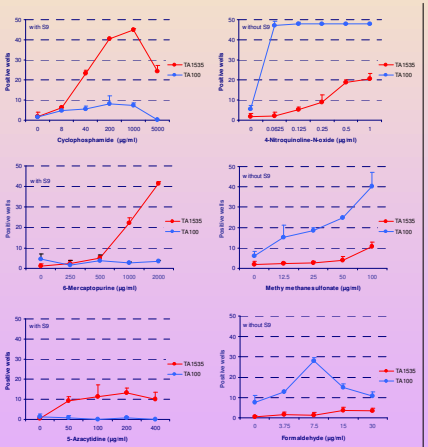


Figure 2:

Ames MPF™ basepair strains: Comparison of TA1535 (hisG46) and TA100 (hisG46[pKM101])

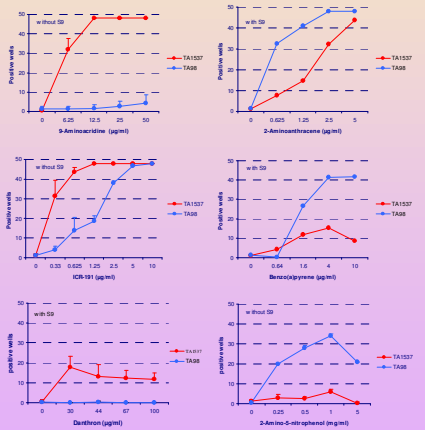


Figure 3:

Ames MPF™ frameshift strains: Comparison of TA98 (hisD3052[pKM101]) and TA1537 (hisC3076)

Compound	S9	Ames MPF				Ames plate incorporation (literature)			
		TA98	TA1537	TA100	TA1535	TA98	TA1537	TA100	TA1535
2-nitrofluorene	-	+++	+++	+	-	pos	pos	pos	neg
Pyrene	- +	- +	+ ++	- ?	- -	neg ?	neg ?/pos	neg ?	neg neg
Benzo(a)pyrene	+	+++	++	+++	-	pos	pos	pos	neg
1,6-dinitropyrene	-	+++	+++	+++	-	pos	pos	n.t.	n.t.
Pyrene-1,6-quinone	-	+++	+++	++	-	n.t	n.t	n.t.	n.t.
Anthracene	-/+	-	-	-	-	neg	neg	neg	neg
9,10-dimethylanthracene	- +	+++ +++	++ ++	++ ++	- -	pos	pos	pos	neg
2-aminoanthracene	+	+++	+++	+++	+++	pos	pos	pos	pos
Methyl methanesulfonate	-	-	-	+++	+	neg	neg	pos	pos/neg
4-nitroquinoline-N-oxide	-	+	+	+++	++	pos	pos	pos	pos
Cyclophosphamide	+	-	-	+	+++	neg	neg	pos	pos
5-azacytidine	+	+	-	-	+	neg	neg	neg/?	pos
6-mercaptopurine	- +	- -	- -	- -	+ +++	neg	neg	neg	pos
ICR-191	-	+++	+++	++	+	pos	pos	pos	neg
9-aminoacridine	-	-	+++	-	-	neg	pos	neg	neg
Proflavin	- +	+ +++	+++ +++	+ ++	- -	? pos	pos pos	neg pos	neg neg
Danthron	+	-	++	-	-	neg	pos	neg	neg
2-amino-5-nitrophenol	-	+++	+	-	-	pos	pos	w-pos	neg
N4-aminocytidine	-	-	-	+++	+++	neg	neg	pos	pos
Formaldehyde	-	+	-	++	-	pos	neg	pos	neg
Na-azide (3h exposure)	-	-	-	++	++	neg	neg	pos	pos
Ethylenediamine	-/+	-	-	-	-	neg	neg	neg	neg/pos
Primidone	-/+	N/A	N/A	-	-			neg	pos
Acetaldehyde oxime	-/+	N/A	N/A	-	-	neg	neg	neg/?	neg/pos

Table 1: Relative mutagenic potential of reference compounds as detected by Ames MPF™ 98, 100, 1535, and 1537. Comparison with published results in the Ames plate incorporation assay.

Scoring: Number of wells with revertant bacteria: +++ = 30 - 48; ++ = 15 - 29; + < 15 ≥ 2-fold induction over baseline

Concordances Ames MPF™ and Ames plate incorporation (from literature; conflicting results have been ignored):

TA98: 95% (19/20); TA1537: 100% (20/20); TA100: 95% (18/19); TA1535: 89% (17/19)

Conclusions:

The new Ames MPF™ assays allow to take advantage of the colorimetric microplate format while using the same *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 that are used in the Ames plate incorporation test. The 384-well microtiter format requires about 6x less test compound and consumables, and considerable less hands-on-time.

The results confirm the usefulness of the liquid microplate format for bacterial mutagenicity testing and expand the range of available *S. typhimurium* strains.

The use of TA1535 and TA1537 allows the detection of additional mutagens compared to the use of TA100 and TA98 only.

Excellent concordances of 100 % (TA1537) 95% (TA98, TA100) and 89% (TA1535) between the microplate and the plate incorporation format were obtained.

The Ames MPF™ assays are therefore a rapid time- and resource-effective pre-registration alternative to the Ames plate incorporation assay using the same strains of *S. typhimurium*.

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