THE AMES MPF™ PENTA I ASSAY:

Mutagenicity Testing in Liquid Microplate Format Using OECD Guideline 471 Compliant Strains *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E.coli* WP2 *uvrA* plus *E.coli* WP2 [pKM101]

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

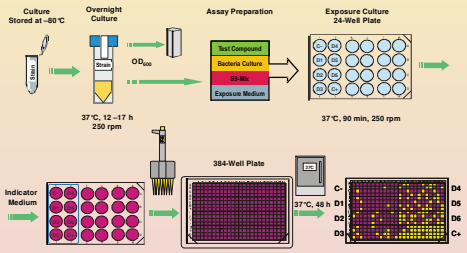
Introduction:

The necessity of testing compounds for genotoxic liabilities is constantly increasing. In drug discovery, genotoxic substances should be removed from further development as early as possible, often at stages where very limited quantities are available. But also the testing of environmental samples, or new regulatory requirements (REACH) for re-testing of existing chemicals increase the need for higher throughput mutagenicity assays. We have earlier introduced the liquid Ames II and Ames MPF (microplate format) assays, which have the advantage of requiring less test compound, consumables and hands-on-time. We are now able to offer in this format all strains required by the OECD guideline 471 for Testing of Chemicals.
The complete bacterial reverse mutation test includes at least five tester strains. *S. typhimurium* TA98, TA100, TA1535 and TA1537 are already successfully used in the microplate format. These 4 tester strains have GC base pairs at the primary reversion site and may therefore not detect certain classes of chemicals. A tester strain with an AT base pair at its primary reversion site was until now not available in the microplate format.
The mutagenic response to 13 reference compounds, including streptonigrin, mitomycin C, aldehydes oxidizing agents and hydrazines, was examined in *E.coli* WP2 *uvrA* and *E.coli* WP2 [pKM101]. The two strains had different sensitivities towards different mutagens. When combined during exposure as "*E.coli* Combo", it was always the more sensitive strain that dominated the response. When compared with published plate incorporation data the results were found to be identical.
The new Ames MPF *E.Coli* Combo assay was combined with the Ames MPF 98/100/1535/1537 assay to create the Ames MPF PENTA I test which meets the strain requirements of the OECD guideline 471. The new Ames MPF PENTA I assay kit is based on the fluctuation method using a preincubation procedure of 90 minutes. The use of a liquid format and 384-well microplates offers a time- and cost effective alternative to the plate incorporation test. As both formats use the same tester strains, results can be compared with existing data sets. The new assay kit includes ready-to-use media and quality controlled bacteria, and allows for high throughput testing.

Test method:

The Ames MPF™ assays are performed in 384-well plates with the histidine auxotroph *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537, and the tryptophan auxotroph *E.coli* tester strains WP2uvrA plus WP2[pKM101]. 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 the required amino acid is added. Each well of the 24-well plate is distributed into 48 wells of a 384 well-plate and incubated for two days to allow revertant bacteria to grow. Mutagenicity is measured by a color change from purple to yellow (pH drop due to bacterial metabolism).

The experiments presented here were done with 3-6 concentrations.



Results:

Table 1: Reference compounds as detected by the Ames MPF™ E.coli *uvrA*, Ames MPF™ E.coli pKM101 and Ames MPF™ E.coli Combo assay. Comparison with published results in the Ames plate incorporation assay.

Compound	S9	Ames MPF uvrA	Ames MPF [pKM101]	Ames MPF Combo	Ames Plate Incorporation uvrA	Ames Plate Incorporation [pKM101]	Ames Plate Incorporation uvrA[pKM101]	Ames Plate Incorporation TA102
Cumene hydroperoxide	-	+	+	+		+	+	
Formaldehyde	-	-	+	+		-/+	+	
Mitomycin C	-	-	+	+		+	-	
Streptonigrin	-	+	+	+		+	+	
Danthron	+	-	-	-		-	-	
Glutaraldehyde	-	-	+	+		+	+	
N4-aminocytidine	-	+	+	+	+			
Hydrazine sulfate	-	+	+	+				+
4-nitroquinoline-N-oxide	-	+	+	+		+	+	
Methyl methanesulfonate	-	+	+	+		+	+	
	+	+	+	+		+	+	
2-aminoanthracene	+	+	+	+		+	+	
2-nitrofluorene	-			-		-	-	
9-aminoacridine	-			-	-			

Figure 1:

Ames MPF™ *E.coli* strains WP2 uvrA and WP2[pKM101] exposed individually or combined ("Ames MPF Combo") to 11 mutagens

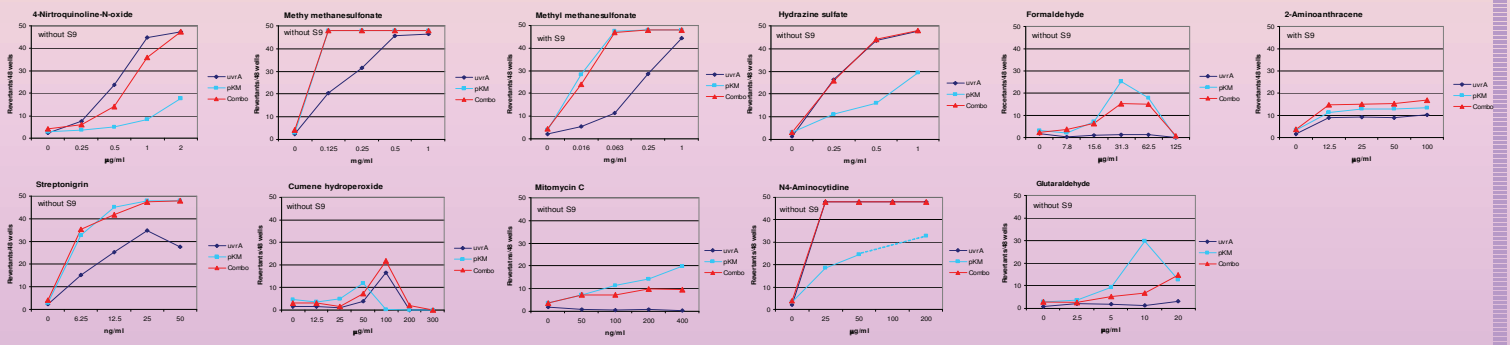
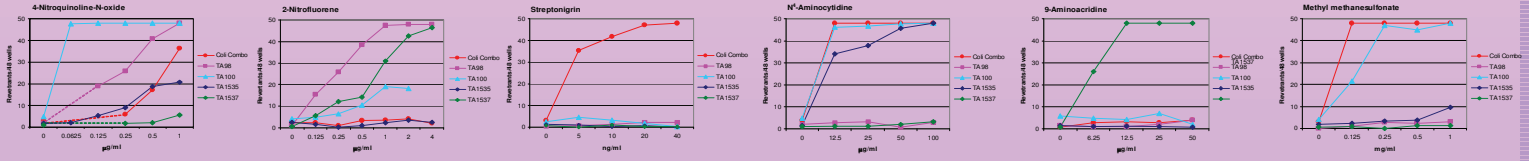


Figure 2:

Ames MPF™ PENTA I Assay: Performance of different strains in the presence of 6 reference compounds



Conclusions:

The new Ames MPF™ PENTA I assay allows to take advantage of the colorimetric microplate format while using the same *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *E.coli* WP2 *uvrA* plus WP2[pKM101] that are used in the Ames plate incorporation test. The 384-well microtiter format requires about 3x less test compound, and considerable less consumables and hands-on-time.

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

The use of E.coli WP2 uvrA plus WP2[pKM101] in the Ames MPF™ PENTA I assay allows the detection of additional mutagens compared to the use of the *S. typhimurium* strains only.

The Ames MPF™ PENTA I assay is therefore a rapid time- and resource-effective alternative to the Ames plate incorporation assay using the strains of *S. typhimurium and E.coli* mentioned in the 'OECD Guideline 471 for Testing of Chemicals'.

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ANIARA DIAGNOSTICA LLC
+1 (513) 770-1991 | +1 (866) 783-3797
7768 Service Center Drive
West Chester, OH 45069
info@aniara.com