The AMES II™ Mutagenicity Assay: An International Validation Study Performed With Nineteen Coded Compounds

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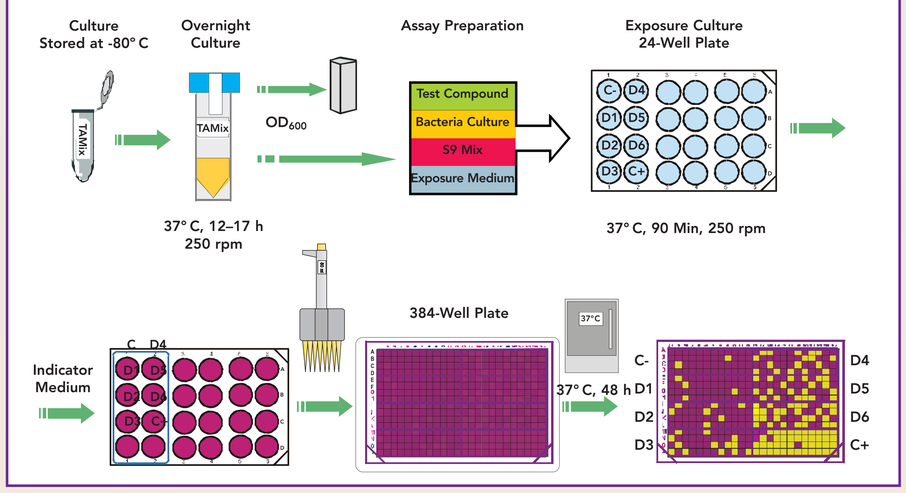
Introduction

The Ames II™ assay, a liquid microtiter modification of the standard Ames plate incorporation test («Ames I»), was used for an international collaborative study with nineteen coded chemicals. The goal of this study was to (1) validate the Ames II as a suitable alternative screening assay to the Ames I test, and (2) to test the Ames II™ assay system for its reproducibility among 9 different laboratories.

Test Method

The Ames II™ assay is performed with the histidine auxotroph *Salmonella typhimurium* tester strains TA98 (frameshift mutations) and TAMix (base-pair substitutions). TAMix is a mixture of six base-pair strains, TA7001-TA7006 in equal proportions, each of which reverts by only one specific base substitution (Ref.1).
The test is performed in microtiter plates. Tester strains and media are available at Xenometrix by Endotell GmbH. Chemical treatment is performed in 24-well plates (6 concentrations in triplicate, concurrently with solvent and positive controls) in the absence and presence of S9 mix. After treatment, a medium containing a pH indicator and lacking histidine is added. Each well of the 24-well plate is then 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 (Fig. 1).

Fig. 1: Ames II Assay Procedure



The Chemicals

- The 19 chemicals selected from the literature (Ref. 2) included known mutagens, non-mutagens and compounds producing conflicting results in the standard Ames plate incorporation test. If possible, chemical pairs were chosen, i.e. carcinogens and non-carcinogens with closely related chemical structure (Table 1).
- The chemicals were coded at random and distributed among nine independent laboratories to allow for an inter-laboratory comparison of the Ames II test system.
- Each chemical was tested by 4-6 investigators.

Table 1 Test Chemicals

Code No.	Chemical	Abbreviation	CAS No.	MW[a]	Mutagenicity[b]
4	2-Acetylaminofluorene	2AAF	53-96-3	223.3	+
13	3-Amino-1,2,4-triazole	Atrz	61-82-5	84.1	-
18	Anthracene	Anth	120-12-7	176.2	-
12	Azoxybenzene	AzoxB	495-48-7	198.2	?
10	Benzidine	Bzd	92-87-5	184.2	+
3	Benzo(a)pyrene	B(a)P	50-32-8	252.3	+
1	Cyclophosphamide	Cphos	6055-19-2	279.1	+
14	Diethylstilbestrol	DES	56-53-1	268.3	-
6	9,10-Dimethylanthracene	DMAnth	781-43-1	206.3	+
8	Diphenylnitrosamine	DphNO	86-30-6	198.2	?
17	Isopropyl N(3-chlorophenyl) carbamate	IsoPC	101-21-3	213.7	-
19	L-Methionine	Met	63-68-3	149.2	-
5	4,4-Methylene-bis(2-chloroaniline)	MOCA	101-14-4	267.2	+
2	2-Naphthylamine	2Naph	91-59-8	143.2	+
7	4-Nitroquinoline-N-oxide	4NQO	56-57-5	190.2	+
11	Pyrene	Pyr	129-00-0	202.3	?
16	D-Sucrose	Sacch	57-50-1	342.3	-
15	Tetramethylbenzidine	TMB	54827-17-7	240.4	-
9	Urethane	Urthne	51-79-6	89.1	?

[a], molecular weight
[b], assessment according to the ICPESTTC study (Ref. 2)
+, positive; -, negative; ?, equivocal

Results

The present Ames II study revealed an overall agreement of 84% with the standard Ames plate incorporation test (Ames I, Fig. 2).

- No false positive results were obtained
- All mutagenic chemicals selected were correctly identified with the Ames II™ assay (e.g. Fig. 3), except cyclophosphamide (Cphos) which was Ames II positive in only 4 of 6 laboratories.
- Two of the compounds with equivocal results in the Ames I test (pyrene, Pyr and diphenylNitrosamine, dPhNO) were weakly but consistently positive in the Ames II test, whereas one (azoxybenzene, AzoxB) produced also conflicting results in the Ames II test.

  Table 2 summarizes the Ames II results obtained by the different participating laboratories.
- All laboratories agreed to 100% in 15 of 19 chemicals (individual questionable results are ignored). Furthermore, all except one laboratory came to the same conclusion for 17 out of 19 test compounds which results in an inter-laboratory consistency of 89.5%.

Fig. 3: Ames II test results with 2-naphthylamine

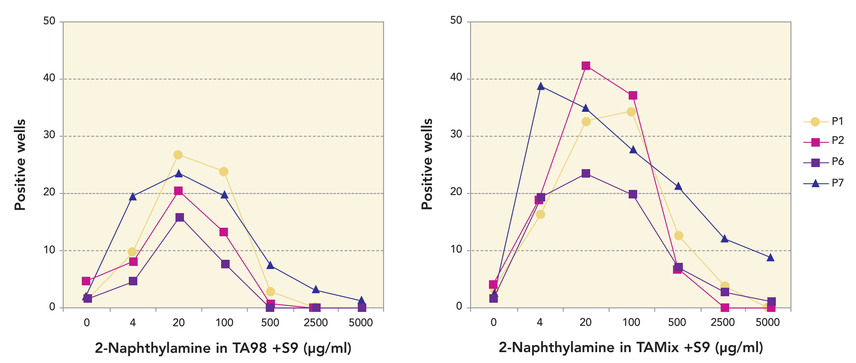


Fig 2: Relative Performances of the Ames I and the Ames II
responses have been normalized (%) because of different group sizes; questionnable response has been ignored

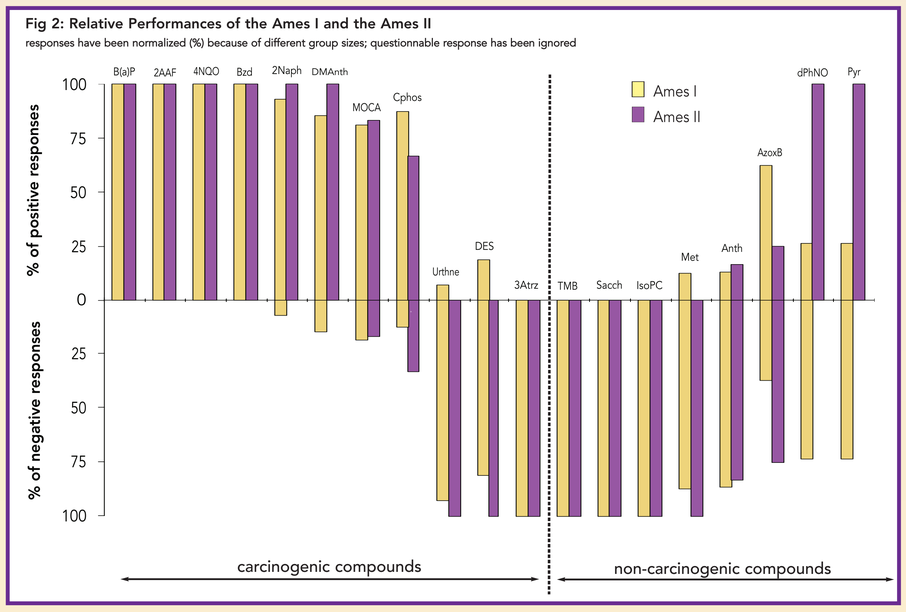


Table 2: Inter-laboratory Consistency

Participant	CODE # 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
P1	pos	pos	pos	pos	pos						pos	equ	neg	neg	neg				
P2	neg	pos	pos	pos						pos					neg	neg	neg	neg	neg
P3			pos	pos	pos	pos	pos						neg	neg	neg	neg	neg		
P5				pos	neg	pos	pos	pos						neg	neg	neg	neg	neg	
P6		pos	pos	pos	pos	pos						neg	neg	neg	neg	neg			
P7	pos	pos						pos	neg	pos	pos	pos	equ					neg	neg
P8						pos	pos	pos	neg	pos	pos					neg	neg	neg	neg
P9	pos				pos	pos	pos	pos	neg		pos			neg	neg		neg	pos	
P1 (Robot)	pos	pos	pos	pos	pos						pos	neg	neg	neg	neg				
P4 (Robot)	neg						pos	equ	neg	pos	equ	neg					neg	neg	neg
Ames I Literature	pos	pos	pos	pos	pos	pos	pos	equ	equ	pos	equ	equ	neg	neg	neg	neg	neg	neg	neg

P = Participating company; pos = positive result; neg = negative result; equ = equivalent result

Conclusion

This study shows that the Ames II™ Assay is a well reproducible test alternative to the traditional Ames test (Ames I) and that the sensitivity of both test systems (Ames I and AmesII) is comparable, **making the Ames II Mutagenicity™ Assay a cost-effective pre-regulatory screening test.**

Advantages of Ames II over Ames I:

- Higher speed format – Microplate format - Automatable
- TAMix allows to record all possible base-pair substitutions in one culture
- Ready to use reagents – Less hands on time
- Colorimetric assay
- Substantially lower consumption of test chemical and plasticware

References

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[2] B.A. Bridges, D. MacGregor, E. Zeiger. Summary report on the performance of bacterial mutation assays. In: Progress in mutation research Vol. 1. Evaluation of short-term tests for carcinogenesis. Report of the international collaborative program, F.J de Serres, J. Ashby (Eds.). Elsevier/North Holland (1981) pp. 49–67

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