NanoAmes: A new tool to evaluate the mutagenic potential of chemicals and impurities using very few micro quantities (µg/ng)

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Introduction

Ames test is the gold test standard in genetic toxicology battery of tests. Ames test is used in regulatory studies (Ames *et al.*, 1975[1]; Gatehouse *et al.*, 1990[2], OECD (1997) [3]) but also the miniaturized Ames test is widely used for screening approach Burke DA, et al.1996 [4] or in GTI strategy by pharmaceutical companies. The major limitation of the Ames test is the needed quantity of compound to be tested, around at least 300 mg by experiment in regulatory Ames test and 20 mg for the miniaturized Ames test. On the other hand, the analysis of test item in different stages of development shows unknown impurities or degradation of test item in few quantity (micrograms to nanograms).

Objective

NanoAmes is an adaptation of the regular Ames test in order to reduce the needed quantity of compound by retaining the design of the regulatory studies: 5 strains with and without metabolic activation.

Methods

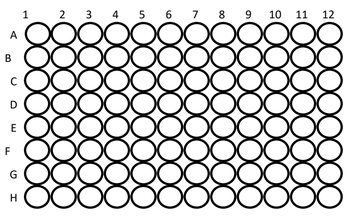
The following was added in each well

Petri dish method

- ✓ strain culture in GenCar medium
- ✓ compound (50µg/well corresponding to 5000µg/plate*) or negative or positive control (2.5 to 500 ng)
- ✓ If appropriate S9-mix

25-well plate method

- ✓ strain culture in GenCar medium
- ✓ compound (2.5µg/well corresponding to 5000 µg/plate*) or negative or positive control (6.25 to 200 ng)
- ✓ If appropriate S9-mix



Incubation 10+/-12h at 37°C

Each well was transfered in tube containing:
- ✓ 2 mL of molten soft agar
- ✓ 500 µL of phosphate buffer pH 7.4

Each tube was pourred in Petri dish containing 20 mL of Minimal Vogel-Bonner Agar

Each well was transfered in tube containing:
- ✓ 3 mL of molten soft agar
- ✓ 720 µL of phosphate buffer pH 7.4

650µL of each tube was pourred in five wells of 25-well plate containing 2mL of Minimal Vogel-Bonner Agar

Incubation 48h or 72h at 37°C

*Highest dose level recommended by OECD 1997

Colonies were counted manually

Materials

✓ Strains:

Six strains of *Salmonella typhimurium* bacteria (TA97a, TA98, TA100, TA1535, TA1537 and TA102) were used in this study. All the tester strains were originally obtained from B.N. Ames (University of California, Berkeley, CA, USA). Bacteria were cultured at 37±1°C for 12 ± 2 hours in growth medium GenCar, containing ampicillin (TA97a, TA98, TA100) or ampicillin and tetracycline (TA102) as appropriate, to provide bacterial cultures in the range of 5.10^8 to 5.10^9 cells/mL.

✓ Positive controls:

Chemical [a]	Dose per well (ng)		Strain(s)	S-9
	Petri dish method	25-well plate method		
2-nitrofluorene (2NF)	25	25-12.5-6.3	TA98	–
Sodium azide (SA)	20	50-25-12.5	TA100, TA1535	–
9-aminoacridine (9AC)	500		TA1537	–
		200-100-50	TA97a	–
Mitomycin C (MC)	2.5	50-25-12.5	TA102	–
Benzo[a]pyrene (B[a]P)	50		TA98	+
2-aminoanthracene (2AA)	20	100-50-25	TA100, TA1535, TA1537	+
		100-50-25	TA98	+
	100	300-150-75	TA102	+

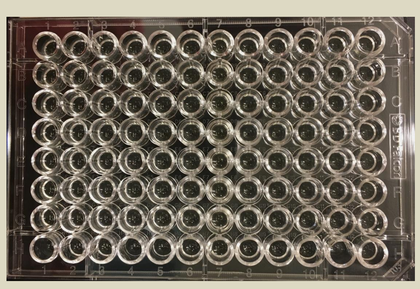
a Obtained from Sigma-Aldrich. MC
Obtained from Alfa-Aesar. 2NF; SA; 9AC; B[a]P; 2AA

Quantity of compound required

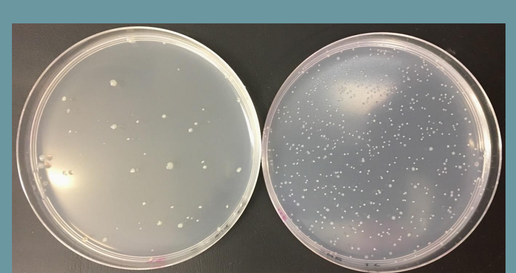
Regulatory Ames test : 300 mg → **NanoAmes Petri dishes: 3 mg**

Miniscreen Ames : 25 mg → **NanoAmes 25-well plates : 35 µg**

96-well plate

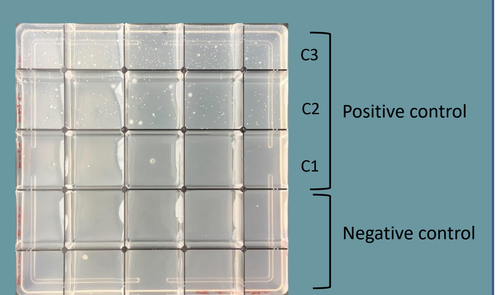


Petri-dish



Negative Control Positive Control

25-wells Petri Dish



Petri dish method				
S9-mix	Strain	Negative control	Positive control	Ratio
Without	TA98	10 (9-46)*	135	13.5
	TA100	143 (61-178)*	321	2.2
	TA1535	7 (5-29)*	140	20.0
	TA1537	7 (3-22)*	620	91.9
	TA102	276 (258-570)*	568	2.1
With	TA98	11 (10-54)*	60	5.7
	TA100	79 (60-185)*	171	2.2
	TA1535	9 (7-25)*	404	46.6
	TA1537	14 (2-23)*	To be added	
	TA102	358 (292-676)*	728	2.0

25-well plate method				
S9-mix	Strain	Negative control	Positive control	Ratio
Without	TA98	2 (0-7)*	10**	5.9
	TA100	18 (7-38)*	67**	3.7
	TA102	47 (16-40)*	179**	3.8
	TA97a	13 (8-27)*	174**	13.2
With	TA98	2 (0-5)*	48**	31.7
	TA100	9 (10-26)*	33**	3.5
	TA102	45 (18-44)*	163**	3.6
	TA97a	6 (16-35)*	40**	6.4

* GenEvolutioN historical data (individual data)

**Doses per well retained :
- -S9 : TA98 25ng, TA100 50ng, TA102 12.5ng, TA97a 50ng
- +S9 : TA98 25ng, TA100ng, TA102 150ng, TA97a 50ng

Conclusion

The revertant colonies number in 6 *Salmonella typhimurium* tester strains recommended by OECD [1][2][3] (TA 97a, TA98, TA100, TA1535, TA1537 and TA102) with negative and positive controls were similar to those found in the regular Ames test. The positive controls showed a response in accordance with the acceptance criteria with this new method.

According to Burke and al. publication [4], volumes of all components can be adjusted and plating performed in a 25-well plate before 72h incubation at 37°C and counting.

References :

[1] Ames B N, McCann J and Yamasaki E (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Research 31, 347-364

[2] Gatehouse D G, Wilcox P, Forster R, Rowland I R and Callander R D (1990). Bacterial mutation assays. In "Basic Mutagenicity Tests UKEMS Recommended Procedures". Report of the UKEMS Sub-committee on Guidelines for Mutagenicity Testing. Part I Revised. Ed D J Kirkland. Cambridge University Press, pp 13-61

[3] OECD (1997). "Bacterial Reverse Mutation Test", in: OECD Guideline for the Testing of Chemicals, Test Guideline 471

[4] Burke DA, Wedd DJ and Burlinson B (1996) Use of the Miniscreen assay to screen novel compounds for bacterial mutagenicity in the pharmaceutical industry Mutagenesis vol.11 no.2 , 201-205.