ASSESSMENT OF A SCREENING EXPERIENCE WITH THE AMES II™ TEST AND FUTURE PROSPECTS

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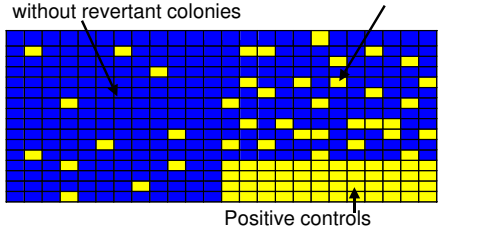
INTRODUCTION

The Ames II™ test, a liquid fluctuation version of the *Salmonella* mutagenicity assay, provided by Xenometrix GmbH, was used for an early compound selection in the discovery process. The aim of this work was to validate the Ames II compared to the standard Ames test and to explore a way to reduce the required compound quantity without lowering the predictability of the test.

MATERIALS and METHODS

This test is composed of a mixture of 6 *Salmonella typhimurium* strains: *TA7001, TA7002, TA7003, TA7004, TA7005* and *TA7006*, which revert to histidine autotrophy by a specific base substitution in the histidine operon. This "mix" is used as a single strain. In addition, the frameshift tester strain *TA98* is used. The mix and *TA98* strains are inoculated in the medium for growth overnight at 37°C. The treatment, performed in 24-wells microtiter plates, allows partial automation and consequently requires about 60-fold less compound (50 mg) than the standard Ames. After a 90 min-incubation treatment with or without Aroclor-induced S9 mix, concurrently with solvent and positive controls, an indicator medium lacking histidine is added to each well. Each well is then aliquoted into 48 wells of a 384-well plate. Within two days, revertants to *His* grow into colonies. The metabolism of the bacterial colony reduces the pH of the medium, changing the colour of the wells. The number of discoloured wells are counted for each concentration and compared to the solvent control (Fig.1). Each concentration is evaluated in triplicate to allow statistical analysis. 350 compounds were tested and three modified conditions were also evaluated to reduce the compound use, namely: test with one strain only, with S9 mix only or with lower concentrations.

Fig.1: 384-well plate



Tab.1: Results of a collaborative study by Xenometrix GmbH (March 2003)

Company	Compound Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
C1	P	P	P	P	P	-	-	-	-	-	P	N	N	N	N	-	-	-	-
C1 (Robot)	P	P	P	P	P	-	-	-	-	-	P	N	N	N	N	-	-	-	-
C2	N	P	P	P	-	-	-	-	-	P	-	-	-	-	N	N	N	N	N
C3	-	-	P	P	P	P	P	-	-	-	-	-	N	N	N	N	N	-	-
C4 (Robot)	N	-	-	-	-	-	P	E	N	P	E	N	-	-	-	-	N	N	N
C5	-	-	-	P	N	P	P	P	-	-	-	-	-	N	N	N	N	N	-
C6	-	P	P	P	P	P	-	-	-	-	-	N	N	N	N	N	-	-	-
C7	P	P	-	-	-	-	-	P	N	P	P	N	N	-	-	-	-	N	N
C8	-	-	-	-	-	P	P	P	N	P	P	-	-	-	-	N	N	P	N
C9	P	-	-	-	P	P	P	P	N	-	P	-	-	N	N	-	N	E	-
Result	?	P	P	P	P	P	P	P	N	P	P	N	N	N	N	N	N	N	N
Ames I	P	P	P	P	P	P	P	?	?	P	?	?	N	N	N	N	N	N	N

N: Negative; P: Positive; E: Equivocal

Sensibility : 83 % nb true positive/total nb positive compounds

Specificity : 100 % nb true negative/total nb negative compounds

Concordance : 89% nb true predictions/total nb compounds

Tab.2: Comparative results between Ames II and Ames I tests (42 proprietary compounds)

Test compounds	Ames I	Ames II™
28	-	-
6	+	+
1	+/-	+
7*	+	-

* 6/7 compounds revert specifically *E. coli* or *TA1535*.

Sensibility : 50 % **Specificity : 100 %**

Concordance : 83 %

Fig.2: Distribution of 56 positive responses among strains:

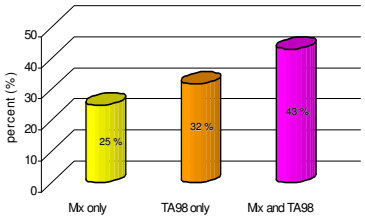


Fig.3: Distribution of 56 positive responses *vs* concentration

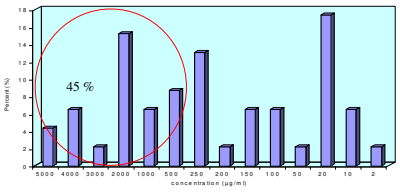
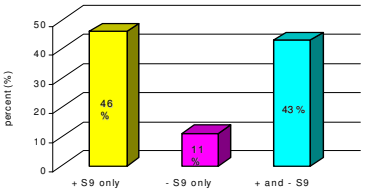


Fig.4: Distribution of 56 positive responses *vs* metabolic activation



RESULTS

350 compounds were tested, including molecules issued from our own research, known non- or genotoxicants, or molecules producing equivocal results. The concordance between the results achieved in this Ames II™ test and those reported in the literature or in the standard Ames test ranged from 79 (Ref.1) to 83% (Tab.2). The concordance reached 89% in a collaborative study (Tab.1). No false positive results were obtained with known non-mutagenic substances. False negative results may arise when chemicals revert only specific strains like *TA1535* or *E. coli WP2 uvrA (pKM101)* which meet no equivalent in the "mix".

The positive responses were randomly distributed among the strains or the concentration range (Fig.2 and 3). In contrast, only 11% of positive results emerged specifically in the absence of S9 (Fig.4), while 89% of genotoxicants should be detected using S9 mix as the only treatment condition.

DISCUSSION - CONCLUSION

Based on the acquired experience on a large number of compounds, the Ames II™ test is a reliable screening tool. When used with the recommended conditions by the supplier, it allows an early identification of genotoxicants, otherwise likely discarded at a later stage of development. The two proposed strains (mix and *TA98*) as well as a high level of tested concentrations are essential to keep an acceptable level of predictability. However, as the compound availability is always of high concern at a screening stage, it is possible to reduce by half the required quantity to be tested (*i.e.* 25 mg) when performed with the metabolic activation as a unique treatment condition. In that case, the number of false negative would be increased by only 2% (decreased specificity).

REFERENCES

1. Gee P. *et al.* (1998). Mutat. Res., 412: 115-130

Form AA48
09-2011