AMES II ASSAY: RESULTS OF A VALIDATION STUDY

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A) AMES II ASSAY / METHOD

I. TEST SYSTEM

The Ames II assay of Xenometrix is a liquid microtiter modification of the traditional Ames test for the detection of potential mutagens in *Salmonella typhimurium*.

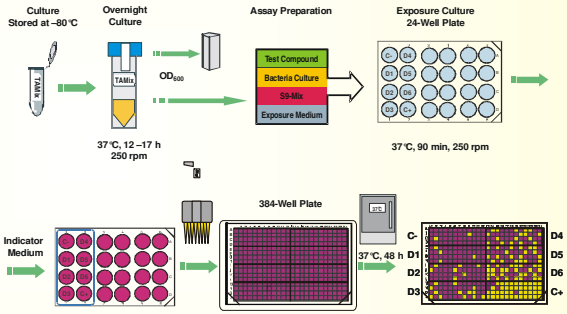
- Media and tester strains, except S9-mix, are available as a kit
- The test is performed in microwell plates
- Mutagenicity (growth of bacteria) is measured colorimetrically from purple to yellow (pH change)
- The Ames II assay uses the so-called "mixed strains" (TAMix) → a mixture of 6 newly developed base-pair strains of the TA7000 series for the detection of base-pair mutations. Each strain will be reverted by only one specific base-pair substitution.
- The Ames II assay is available in two versions:
  - → "Manual kit" (benchtop version for routine analysis)
  - → "High throuput screening (HTS)" (automatable version)

II. TESTER STRAINS

The Ames II assay is performed with the tester strains TA98 (framshift mutations) and TAMix (mixed strains TA7001 - 7006, base-pair substitutions).

STRAIN	MUTATION	TYPE	TARGET	CELL WALL	REPAIR	pKM101
TA98	*hisD3052*	frameshift	GC	*rfa*	*uvrB*	+
TAMix	TA7001 - 7006					
TA7001	*hisG1775*	base-pair	T:A → C:G	*rfa*	*uvrB*	+
TA7002	*hiC9138*	base-pair	T:A → A:T	*rfa*	*uvrB*	+
TA7003	*hisC9074*	base-pair	T:A → G:C	*rfa*	*uvrB*	+
TA7004	*hisG9133*	base-pair	C:G → T:A	*rfa*	*uvrB*	+
TA7005	*hisG9130*	base-pair	C:G → A:T	*rfa*	*uvrB*	+
TA7006	*hisG9070*	base-pair	C:G → G:C	*rfa*	*uvrB*	+

III. ASSAY PROCEDURE/METHOD



IV: ADVANTAGES OF THE AMES II ASSAY

- Routine analysis → compound throughput is ~ 5 times higher with the "Ames II Manual System" than with the traditional Ames test
- Screening (HTS) → ~ 1'000 compounds / year / robot / technician with a partly automated version

V: LIMITATIONS

- At present not applicable for registrations/authorizations of new chemicals/pesticides/drugs
  - → until now no existing guideline
  - → until now no acceptance by the authorities

B) AMES II ASSAY / VALIDATION STUDY

I. AIM

Validation of a high throughput screening version (HTS) of the Ames II assay (= automated version → single experiment without replicates) using selected genotoxic/non-genotoxic compounds.
Comparison with the classical Ames assay (Ames I assay) with regard to:

- Concordance of the results between the two test systems
- Sensitivity (percentage of correctly identified genotoxic/carcinogenic compounds) and specificity (percentage of correctly identified non-genotoxic/non-carcinogenic compounds) of the two test systems

II. TEST COMPOUNDS

- 127 compounds (1st comparison) including different chemical classes were selected according to the criteria listed below:
  - → negative in the traditional Ames assay, possibly positive in other, non-bacterial genotoxicity tests
  - → positive in the Ames plate incorporation assay, partly in different tester strains
  - → positive only when using a modification of the Ames assay (e.g. pre-incubation test, prival modification, liquid suspension assay, addition of norharman etc.)
- For 95 compounds with different genotoxic profiles there are sufficient additional in vitro- and/or in vivo data to allow an assessment for genotoxicity (2nd comparison)
- For 70 compounds there are sufficient data to allow an assessment for carcinogenicity (3rd comparison)

III. RESULTS

1. COMPARISON OF THE TWO AMES TEST SYSTEMS: RESULTS OBTAINED WITH 127 COMPOUNDS

AGREEMENT ca. 75%		AMES II ASSAY negative	AMES II ASSAY positive
AMES I ASSAY	negative	41 (32.3%)	16 (12.6%)
	positive	16 (12.6%)	54 (42.5%)

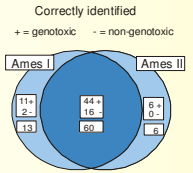
2. COMPARISON OF THE RESULTS OF THE TWO AMES TEST SYSTEMS: GENOTOXICITY DATA (95 COMPOUNDS)

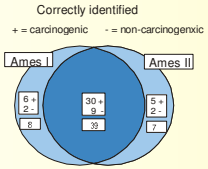
	AMES I ASSAY	AMES II ASSAY
SENSITIVITY[1]	55/75 73.3%	50/75 66.7%
SPECIFICITY[2]	18/20 90.0%	16/20 80.0%
ACCURACY[3]	55+18 = 73/95 76.8%	50+16 = 66/95 69.5%

3. COMPARISON OF THE RESULTS OF THE TWO AMES TEST SYSTEMS: CARCINOGENICITY DATA (70 COMPOUNDS)

	AMES I ASSAY	AMES II ASSAY
SENSITIVITY[1]	36/52 69.2%	35/52 67.3%
SPECIFICITY[2]	11/18 61.1%	11/18 61.1%
ACCURACY[3]	36+11 = 47/70 67.1%	35+11 = 46/70 65.7%

1) = correctly identified positive compounds
2) = correctly identified negative compounds
3) = total percentage of correctly identified compounds





IV. CONCLUSIONS

- The percentage of correctly identified
  - genotoxic/carcinogenic compounds (= *sensitivity*)
  - non-genotoxic/non-carcinogenic compounds (= *specificity*)

  of the two Ames test versions is comparable
- About ¾ of all compounds are correctly identified by both assay systems
- In addition, each assay system correctly detects different compounds (possible reasons: different methodology, different strains, different concentrations of S9-mix)
- The Ames II assay is therefore suitable for the screening of mutagens/genotoxic carcinogens

REFERENCES

Gee, P., Maron, D.M., Ames B.N.
Detection and classification of mutagens: A set of base-specific Salmonella tester strains.
Proc. Nat. Acad. Sci USA, 91, 11606 - 11610 (1994)

Gee, P., Sommers, C.H., Malick, A.S., Gidrol, X.M., Todd, M.D., Burris, R.B., Nelson, M.E., Klemm, R.C., Zeiger, E.
Comparison of responses of base-specific Salmonella tester strains with the traditional strains for identifying mutagens: The results of a validation study
Mut.Res. 412, 115 - 130 (1998)

Gee, P., Schneider, J., Engelhardt, G., Jacob. E.
Evaluation of a screening assay using the Mix (TA7001, TA7002, TA7003, TA7004, TA7005 and Ta7006) and TA98 for mutagenic potential of compounds.
In preparation