

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*.

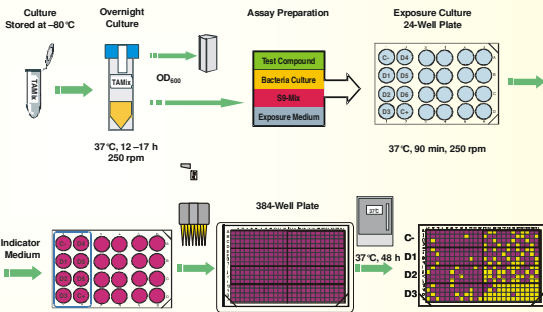
- Media and tester strains, except S9-mix, are available as a kit
- The test is performed in microwell plates
- Mutagenicity (growth of bacteria) is measured colorimetrically from purple to yellow (pH change)
- The Ames II assay uses the so-called "mixed strains" (TAMix) → a mixture of 6 newly developed 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 throughput screening (HTS)" (automatable version)

II. TESTER STRAINS

The Ames II assay is performed with the tester strains TA98 (frameshift 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	hisG9138	base-pair	T:A → A:T	rfa	uvrB	+
TA7003	hisG9074	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	positive
AMES I ASSAY negative	41 (32.3%)	16 (12.6%)
AMES I ASSAY positive	16 (12.6%)	54 (42.5%)

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

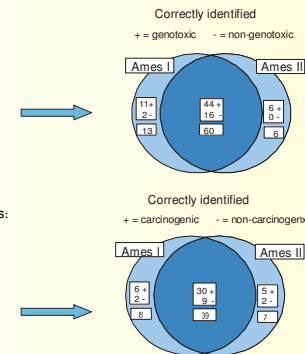
2. COMPARISON OF THE RESULTS OF THE TWO AMES TEST SYSTEMS: GENOTOXICITY DATA (95 COMPOUNDS)

	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



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