

Comparative analysis of miniaturized Ames assay variations for substances with ambiguous testing outcomes



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Introduction

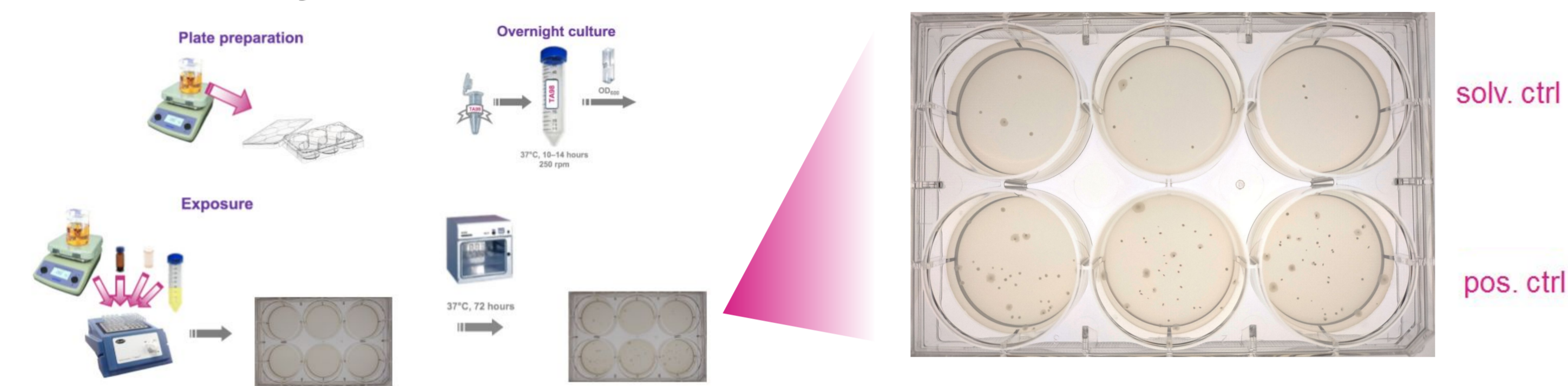
The Ames assay is based on the concept of bacterial reverse gene mutation, and it is proven to be the most widely applied test in **mutagenicity assessment**. Increasing emphasis is placed on the development of **miniaturized versions** of the traditional Ames test with an accentuated goal to **reduce the necessary amount** of test chemicals, reagents, and liver microsomal S9 fraction resulting in a reduction of test animals. Herein we present a **comparative analysis** of the Ames MPF system, the MicroAmes6 assay, results from an OECD draft review paper [1], and the NTP database [2].

Highlights

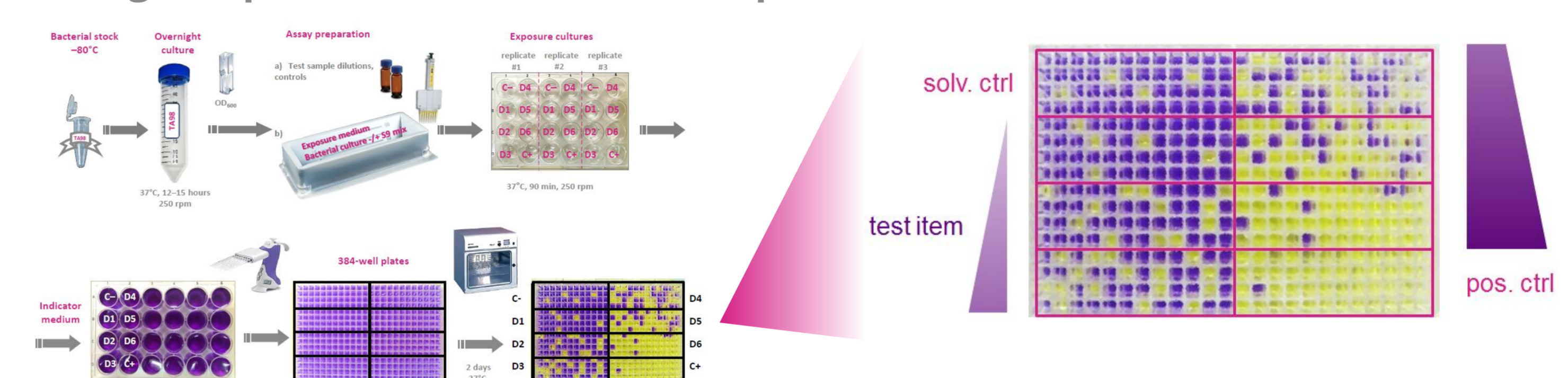
- Miniaturized Ames assays to better support **mutagenicity testing** of chemicals during **early phase development**
- The performance of the miniaturized agar-based Ames test in 6-well plates depends on the **bacterial cell density**
- High level of **concordance** between results acquired with miniaturized Ames assays and the traditional Petri dish test for the selected compounds

Methods

The Xenometrix MicroAmes6 98/100 is an agar-based miniaturized bacterial reversion assay.

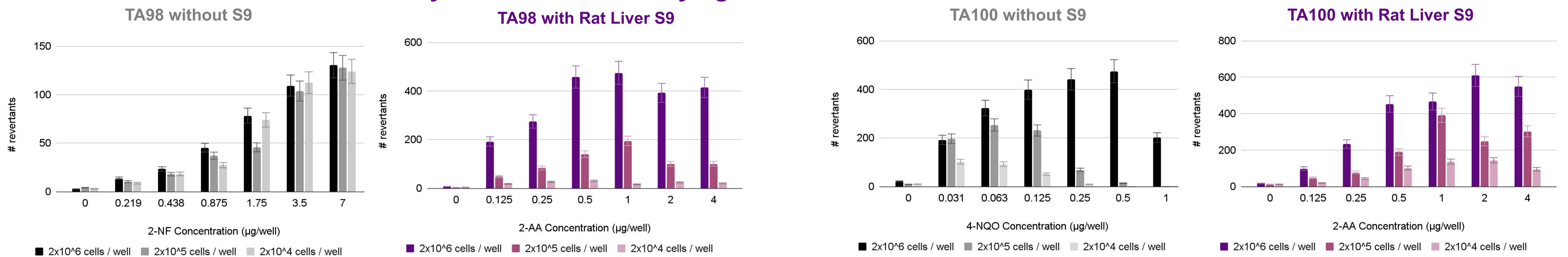


The Xenometrix Ames MPF™ 98/100 is a microplate fluctuation assay to test mutagenic potential of chemical compounds.



Results

Performance of the MicroAmes6 assay as a function of varying cell densities



Mutagenicity profiles identified by miniaturized Ames test systems in comparison with traditional Ames assay results

	(±)-Epichlorohydrin	7,12-Dimethyl benz[a]anthracene	Phenformin HCl	ICR191	N-Nitroso-diphenylamine	6-mercaptopurine	m-Phenylene-diamine
CAS Number	106-89-8	57-97-6	834-28-6	17070-45-0	86-30-6	50-44-2	108-45-2
2D structure							
MicroAmes6	 -ve -ve	 +ve	 -ve	 +ve	 -ve -ve	 -ve -ve	 -ve +ve
AmesMPF™	 -ve -ve	 +ve	 -ve	 +ve	 -ve -ve	 -ve -ve	 -ve
OECD Draft DRP [1]	+ve +ve	+ve	Eq	+ve	-ve -ve	+ve	n.a.
NTP database [2]	-ve -ve	n.a.	-ve	n.a.	-ve -ve	-ve +ve	-ve +ve

Conclusion

The miniaturized Ames tests are instrumental in the assessment of mutagenicity potential of compounds during early phase development. Therefore, the development and subsequent validation of miniaturized bacterial mutagenicity tests is crucial to provide highly reliable, cost- and resource-effective methodologies that can support strategic decision-making across industries. The results indicate that the performance of the agar-based 6-well plate Ames assay is dependent on the applied bacterial cell density, which underlines the importance of investigating such parameters in the course of miniaturized assay development. The work presented here contributes to the mutagenicity assessment of compounds with previously reported ambiguous testing outcomes, and we conclude that the miniaturized agar-based and microplate fluctuation assays can provide results with high level of concordance in comparison to data acquired with the traditional Petri dish-based Ames test. We aim to further corroborate these findings with testing an extended number of selected compounds, the inclusion of other bacterial strains in our testing pipeline, as well as the correlation of our *in vitro* experimental results with *in silico* data.

References:
[1] OECD Draft Detailed Review Paper on the miniaturised versions of the bacterial reverse gene mutation test
[2] National Toxicology Program (NTP) coordinated by United States Department of Health and Human Services

The AmesMPF and MicoAmes6 are proprietary miniaturised Ames assays developed by Xenometrix AG. Check out our homepage xenometrix.ch for further information, and follow us on social media.