JRF GLOBAL

Introducing the Miniaturized Ames Test

JRF is committed to advancement of its abilities and honing our skills to undertake safety evaluation for their sponsors, ensure that the test output is reliable, reproducible and robust.
As part of our continuing efforts to improve and streamline the scientific evaluation strategies to meet the market demand, we're excited to introduce the Miniaturized Ames Test, a compact, efficient, and environmentally sustainable version of the classic Bacterial Reverse Mutation Test, designed to streamline early-stage genotoxicity assessment.

Background

The Bacterial Reverse Mutation Test (Ames Test) is the preliminary *in vitro* assay developed in the 1970s to detect gene mutations, caused by xenobiotic compounds. Even after decades, the Ames test remains to be the gold standard for mutagenecity assesment and has its significance in the genotoxicity test battery, providing early insights into a substance's potential to cause DNA damage and subsequently, its mutagenic and carcinogenic risk.

Why Miniaturized Versions?

Benefits for the New Product Developers

The typical safety evaluation of the library of compounds in their developmental cycle starts with an assessment of their mutagenic potential. At the screening stage, the product development team would like to undertake quick and economical tests, which help them to screen out some of the molecules of the library potentially for their mutagenic potential. Most of the global new product development teams are now increasingly diverting their focus towards efficiency, sustainability, and reduction in resource consumption, which are inevitable factors in this fast-paced age to compete in the ever-growing market. The paradigm shift from the standard Ames test toward a variety of miniaturized formats offers remarkable benefits in this direction.

- Reduced amount of test chemical, solvent and sample volume needed
- Faster throughput
- Cost-effectiveness
- Less space needed and provides enhanced data density

Application Of Miniaturised Ames Test In Different Regulatory Domains

Prediction of mutagenicity in the early stage of development when used in a tiered approach with in silico analysis Testing of impurities Screening of candidate drugs Testing of substances available in low amounts (e.g., intermediates) Identifying genotoxic fraction	**Drugs/ Pharmaceuticals and Complex mixtures**
Food, food contact materials, food additives	Very early screening Testing of impurities/metabolites Testing of substances available in low amounts
Very early screening	**Consumer products (cosmetics and cosmetic ingredients) and New/Existing chemicals**
Agrochemicals, pesticides and biocides	Very early screening Testing of impurities/metabolites Testing of substances available in low amounts

Different Types of Miniaturized Ames Test & A Closer Look at the 6-Well Agar Version

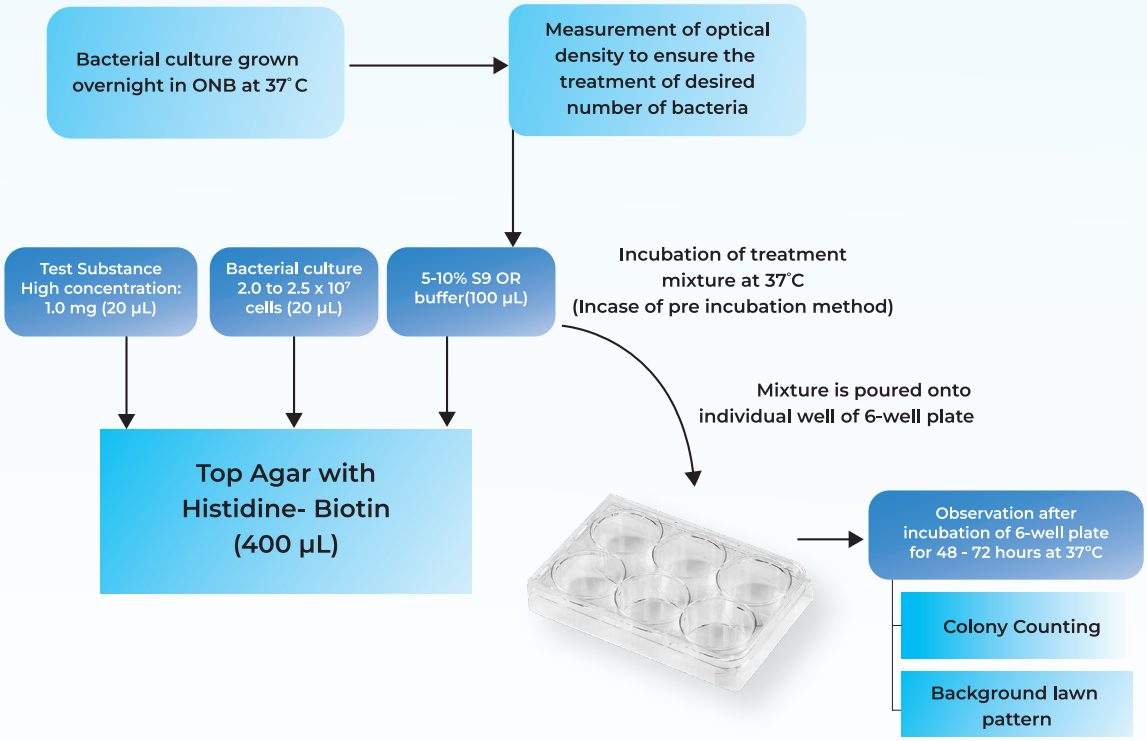
Miniaturized Ames tests come in various formats, each offering unique advantages. These include microfluctuation tests (Ames MPF™ and Ames-II) and agar-based methods (6-well plate and 24-well plate assay).

Among these, the 6-well agar version stands out for its balance of miniaturization with robust performance without changing the basic standard method. Only about 10 – 15 mg of the test item per strain is needed to perform one experiment (5 x times less than the standard test). Moreover, it was the only assay for which enough data are available to conduct a 5-strain overall call analysis (*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA102 or E. coli WP2 uvrA) among other miniaturized formats.

- Both the plate incorporation and the pre-incubation method can be performed in the 6-well plate format
- Nearly 100% concordance in overall mutagenicity judgement compared to the standard test has been reported with high strain-to-strain concordance, i.e., 94%

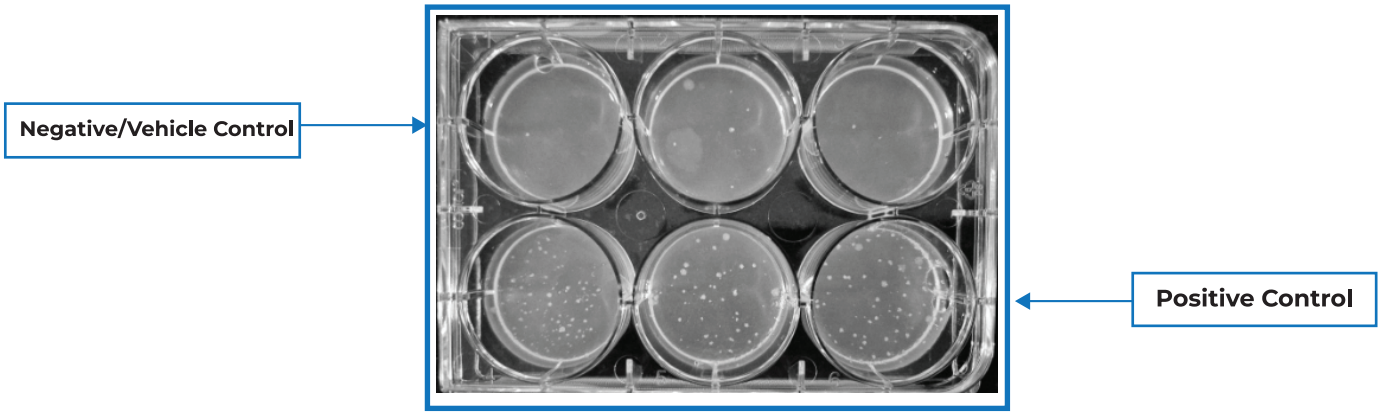
Simplified Procedure of the 6-Well Agar Version of Mini-Ames

The procedure for the 6-well agar version is a streamlined adaptation of the traditional method:



Evaluation

The data sets are judged positive based on the fold-rule (increase in the number of bacterial revertants compared to concurrent negative/vehicle control).



Results

Tester strains	Bacterial Revertant Counts Range			
	Without S9		With S9	
	Negative Control	Positive Control	Negative Control	Positive Control
TA1537	0 - 2	85 - 178	1 - 6	22 - 63
TA1535	5 - 12	41 - 85	1 - 3	15 - 21
TA98	3 - 17	100 - 119	4 - 12	211 - 296
TA100	21 - 32	162 - 271	16 - 40	101 - 296
TA102	15 - 57	144 - 194	10 - 50	93 - 181
E coli WP2 uvrA pKM101	4 - 13	83 - 122	2 - 20	89 - 169

Regulatory Standpoint and Further Inclusion in GLP Criteria (OECD 358- Detailed Review Paper)

For the future use of the miniaturised bacterial reverse gene mutation tests, there are (at least) 3 possible scenarios:

(I) As 100% accepted alternative for the standard bacterial reverse gene mutation test

(II) As an alternative test when the standard bacterial reverse gene mutation test is not an option

(III) Exclusively for screening

The regulatory bodies are now increasingly showing their interest in miniaturized genotoxicity assays. While the miniaturized Ames test is gaining traction for early screening, rigorous validation against established guidelines is required for the test's full regulatory acceptance for GLP-compliant studies. We believe that by establishing this 6-well agar version of the Ames assay at JRF, our efforts will contribute a great value for regulatory decision making. These efforts also contribute towards its broader acceptance to provide a fully compliant and trusted method for safety assessment in various industries, including pharmaceuticals, chemicals, and cosmetics.

Reference

Diehl, M. S., Willaby, S. L., & Snyder, R. D. (2000). Comparison of the results of a modified miniscreen and the standard bacterial reverse mutation assays Environmental and molecular mutagenesis,36(1), 72-77.

Flamand, N., Meunier, J. R., Meunier, P. A., & Agapakis-Caussé, C. (2001). Mini mutagenicity test: a miniaturized version of the Ames test used in a prescreening assay for point mutagenesis assessment.Toxicology *in vitro* 15(2), 105-114.

Mortelmans, K., & Zeiger, E. (2000). The Ames Salmonella/microsome mutagenicity assay. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 455(1-2), 29-60.

Nicolette, J., Dakoulas, E., Pant, K., Crosby, M., Kondratiuk, A., Murray, J., ... & Kolaja, K. (2018). A comparison of 24 chemicals in the six-well bacterial reverse mutation assay to the standard 100-mm Petri plate bacterial reverse mutation assay in two laboratories.Regulatory Toxicology and Pharmacology, 100, 134-160.

OECD, 2020: The Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, OECD 471, Bacterial Reverse Mutation Test, adopted by the Council on July 21, 1997 and corrected on June 26, 2020 (CAS RN parag.24).

OECD, 2022, Detailed Review Paper on the miniaturised versions of the bacterial reverse gene mutation test, OECD Environment, Health and Safety Publications Series on Testing & Assessment No. 358, ENV/CBC/MONO(2022)14, September 19, 2022.

About the author

Vaibhav – Research Officer, Mutagenicity Section with over six years of experience in the field of pre-clinical testing, Vaibhav brings deep expertise in conducting a range of genotoxicity assays, including the Ames test and *in vivo* micronucleus test. He also leads in-chemico sensitization studies, notably the DPRA assay using HPLC. Beyond his core work, Vaibhav has developed strong proficiency in handling radio-labelled compounds, as well as reconstructed 3D RhE and human skin tissues, which play a key role in advancing *in vitro* alternative testing approaches. His hands-on experience and commitment to scientific precision continue to drive innovation within the research team.

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