THE MICROPLATE AGAR - REPLACEMENT, REDUCTION AND REFINEMENT OF THE MICROSUSPENSION SALMONELLA/MICROSOME ASSAY

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Abstract

The Salmonella/microsome assay is the most used mutagenicity test both for evaluation of chemicals and environmental samples. The objective of this study was to miniaturize the microsuspension version of this assay under the concept of the 3R.

Key words:

Mutagenicity, 3R, Ames.

Introduction

The Salmonella/microsome assay is the most used mutagenicity test both for evaluation of chemicals and environmental samples¹. There are several versions of protocols available in the literature²⁻⁴. Miniaturization of toxicological tests has been a tendency in compliance with the concept of the 3Rs (Replacement, Reduction and Refinement). The microsuspension version of this assay is performed with conventional petri dishes (90 x 15 mm) and uses 5X concentrated bacteria and 10 times less sample and S9 mixture. It has been extensively used for environmental samples testing, including in Effect Directed Analysis (EDA). It can be used with diagnostic strains, which have different spontaneous reversion rates. The objective of this study was to miniaturize of the microsuspension Salmonella/microsome assay using agar microplates under the concept of the 3R.

Results and Discussion

Following the same principles of the microsuspension protocol and aiming a reduction of the assay in 10 times. The conventional plates were replaced by microplates with 12 wells, and the method which is illustrated in the Image 1 was refined to correct the problem of using small amounts of sample (less than 2 µL). For validation of the miniaturization, three strains (TA1538, TA98 and YG1041) were selected because of their different spontaneous reversion rates (low, mean and high), and tested with mutagenic chemicals. The microsuspension and miniaturization procedures were made as similar as possible conditions possible, and similar results were obtained when compared (see Chart 2). The advantages of using the miniaturization are presented in the Chart 1.

Image 1. Diagram depicting the steps involved in the miniaturization of the microsuspension protocol.

Sample	4x Less
Metabolic Activation S9)	4x Less
Medium	5x Less
Plates	8x Less

Chart 1. The advantages of using the Microplate Agar.

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Minimum Effective Concentration (MEC₂) ng/µL

Sample	S9	TA1538		TA98		YG1041	
		Kado	MPA	Kado	MPA	Kado	MPA
1NTP	-	0.016	0.013	0.0015	0.0020	0.0002	0.0002
BaP	+	2.0	1.6	3.5	7.3	0.7	0.7
4NQO	-	0.03	0.03	0.03	0.03	0.0007	0.0006

Chart 2. Comparison of the MPA and Microsuspension protocols (Kado) for TA1538, TA98 and YG1041 strains of *Salmonella typhimurium* in the presence (+) and absence (-) of metabolic activation (S9).

Conclusions

MPA and Microsuspension provided similar sensitivity. The MPA is less laborious, uses less sample, materials and reagents. MPA procedure seems to be a promising tool specially to test environmental samples for mutagenic activity when quantity of sample is a limiting factor.

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