GUM Meeting | Poster #31 | 20-22 March 2024 | Kaiserslautern, Germany

Bacterial cell density as a crucial factor for the performance of the miniaturized Ames test systems

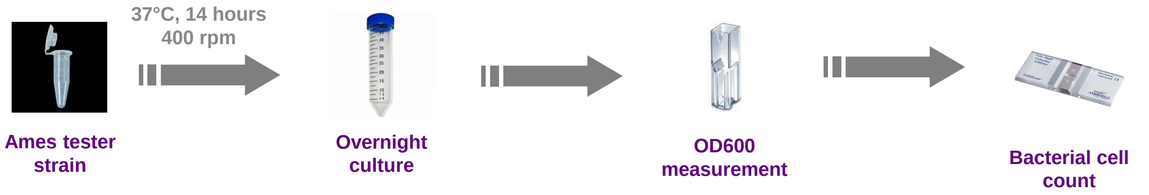
Csaba Boglári[1*], Cécile Koelbert[1]
[1]Xenometrix AG, Gewerbestrasse 25, 4123 Allschwil, Switzerland
* Corresponding author (cbo@xenometrix.ch)

Introduction

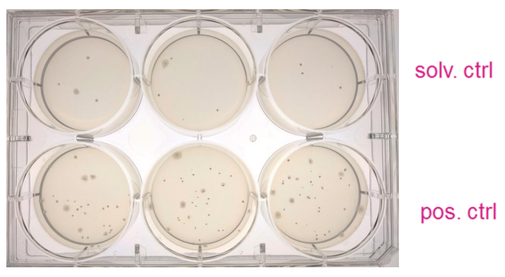
Developing effective methods for testing genotoxic compounds is essential, and the **bacterial reverse mutagenicity test** has long been a cornerstone in genotoxicity assessment. However, numerous scientific and technical challenges must be overcome to tailor solutions suitable for diverse industrial and academic needs. **Miniaturized** versions of the Ames test have emerged as promising alternatives to traditional Petri dish-based assays, offering advantages such as reduced consumption of test materials and supplies, decreased S9 consumption in line with the **3R principles**, as well as streamlined procedures and reduced hands-on time. Additionally, miniaturized Ames test systems enable higher throughput, facilitating the simultaneous testing of multiple compounds and the **early exclusion of DNA-reactive substances during development**. This poster aims to provide a better understanding of **bacterial cell density** as a critical factor influencing the performance of miniaturized Ames assays.

Methods

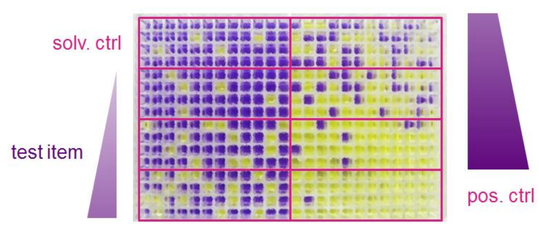
We used overnight cultures of the Ames tester strains followed by 14 hours of incubation at 37°C shaking at 400 rpm. Following the **overnight incubation** the OD600 value was measured and the cell number was determined using a cell counting chamber. The overnight culture was diluted to prepare bacterial suspensions with **different cell densities** serving as an input to the **miniaturized Ames assays**.



The **Xenometrix MicroAmes6** is an agar-based miniaturized Ames test in 6-well plate format. The test substance is considered positive if there is a 2-fold or higher fold induction over the negative control in the number of revertant colonies.

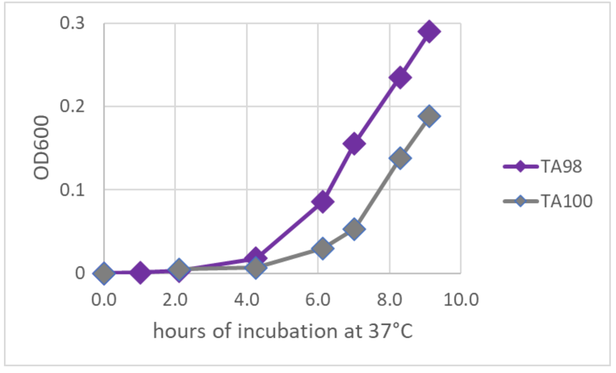


The **Xenometrix Ames MPF™** is a microplate fluctuation assay. The readout is color-based. If there is no reversion event, the well is purple, while the reversion is indicated by yellow coloration.



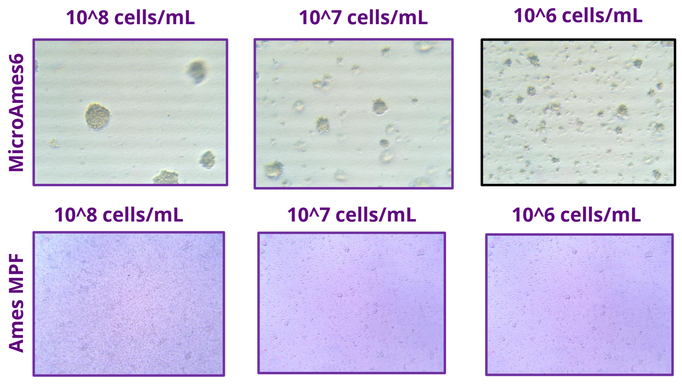
Results

Optimized overnight growth of Ames tester strains



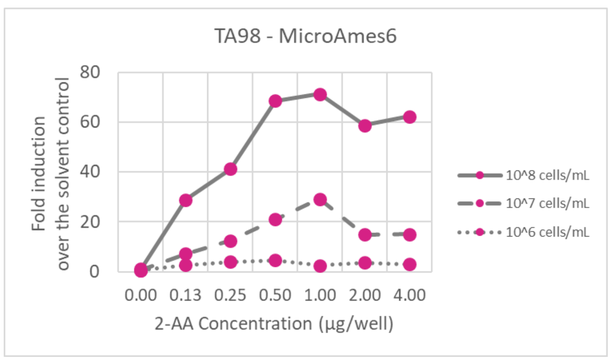
The optical density of Ames tester strains was monitored at various timepoints during the incubation process post-inoculation. A refined growth protocol ensures that the strains reach the appropriate phase at the time of assay, which is essential for obtaining accurate and reproducible assay results. Here we present the growth curves of two Salmonella Ames tester strains: TA98 and TA100.

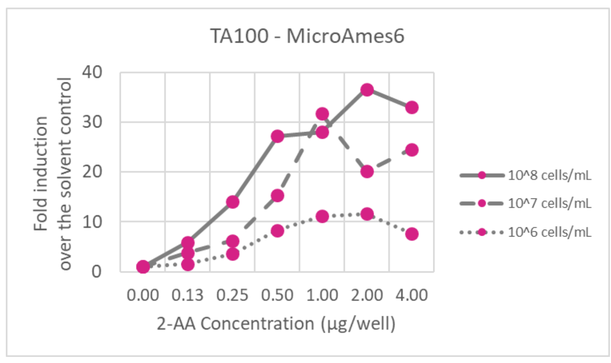
Investigation of the bacterial background lawn

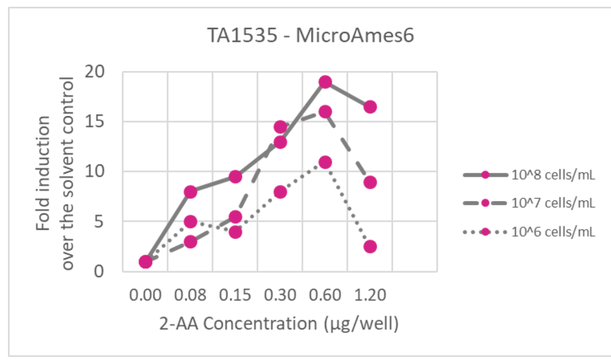


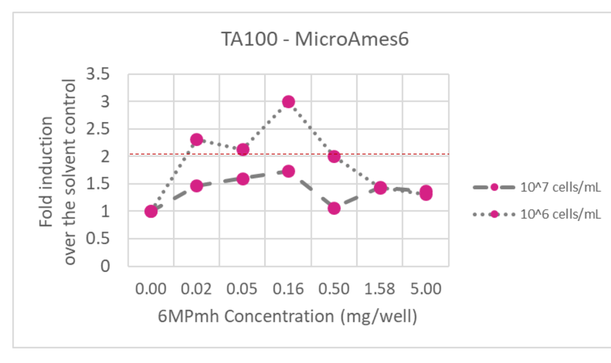
Microscopic images were captured at 250x magnification to observe the bacterial background lawn of a Salmonella Ames tester strain at various cell densities. The negative control wells were examined (vehicle: DMSO). Image capture coincided with the mutagenicity evaluation, occurring either after 72 hours at 37°C for MicroAmes6 or after 48 hours for Ames MPF.

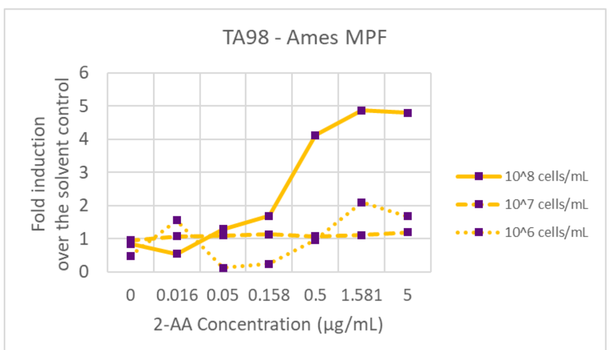
Dose response to known mutagens with different bacterial cell densities in the miniaturized Ames assays

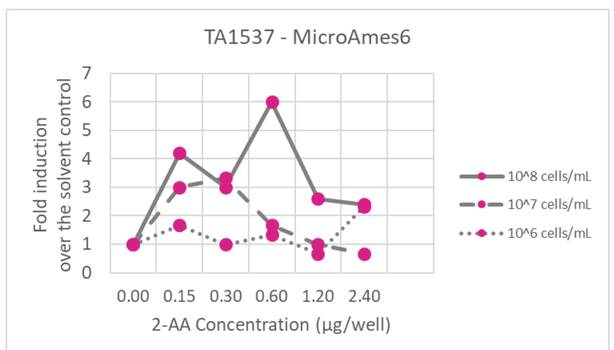


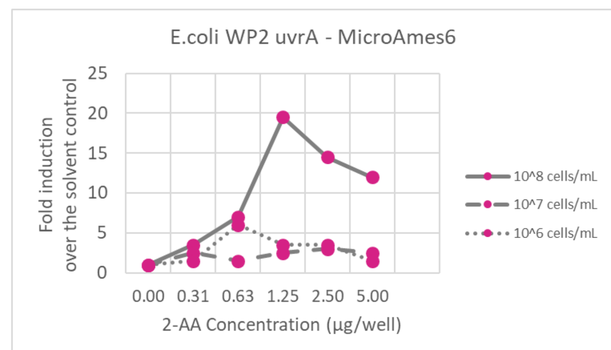












All experiments performed in the presence of 10% or 30% rat liver S9 microsomal fraction in the MicroAmes6 or Ames MPF, respectively. Fold induction over the solvent control in the number of revertants is presented as the function of the varying concentration of the chemicals (2-AA: 2-Aminoanthracene [613-13-8], 6MPmh: 6-mercaptopurine monohydrate [CAS: 6112-76-1]). Input cell density of the bacterial cell culture is represented with continuous or dashed lines on the graphs.

Conclusion

In the vast parameter space of optimizable factors the bacterial cell density in the exposure cell culture plays a crucial role in the miniaturized Ames assays. Here we emphasize that the establishment of a reproducible growth protocol is essential for reproducible and reliable Ames test results. Investigation into the performance, sensitivity, and reproducibility of two miniaturized Ames assays, the liquid microplate format, Ames MPF, and the 6-well plate format, MicroAmes6, was conducted with varying bacterial cell densities for both Salmonella and E. coli Ames tester strains. Our study revealed that MicroAmes6, the 6-well agar plate format is more sensitive to bacterial cell number. We found that in certain cases the sensitivity of the 6-well plate format can be influenced by the cell density of the bacterial cell culture, i.e. compounds can turn positive with lower cell number in this miniaturized Ames assay. We conclude that the microplate fluctuation format, Ames MPF requires 10^8 cells per mL bacterial cell density to be effective and its sensitivity is not reliant on the cell number. These findings contribute valuable insights for optimizing miniaturized Ames tests, especially the 6-well agar plate format and underscore the importance of considering bacterial cell density in assay design and interpretation.

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.