On the limits of miniaturization of the Ames test

Limited availability of test compounds necessitates to miniaturize early pre-clinical toxicity or mutagenicity assays such as the Ames test. We discuss the factors that may be limiting for miniaturized assays such as the liquid microplate versions Ames MPF/Ames II. Upon reducing the number of bacteria a point will be reached where the solvent controls approach zero and thus would no longer allow to calculate a reliable "fold induction". It is concluded that the Ames II / Ames MPF liquid microplate versions of the Ames test achieve an optimal level of miniaturization without compromising reproducibility and sensitivity, particularly towards weak mutagens.

The need for minimizing the consumption of valuable test compounds in early testing protocols has repeatedly led to the question if the Ames MPF/Ames II test can be further miniaturized. The limiting factors are not liquid handling or sensitivity of the read-out, but rather the **inherent spontaneous reversion frequency of the Salmonella strains** used. In order to detect mutagenic compounds the test has to be able to compare induced reversion events with the spontaneous reversion rate, thus allowing to calculate a statistically significant and meaningful "fold induction".

In the classical Ames plate incorporation test the spontaneous number of revertant colonies per plate is in the range of 10-25 with strains TA98, TA1535 and TA1537. TA100 has a higher spontaneous reversion rate of about 100-150 colonies per plate.

In the Ames MPF and Ames II liquid microplate format version of the Ames test the volumes and conditions have been adjusted to yield typically 0-8 (TA98, TA1535, TA1537) and 0-12 (TA100) spontaneous revertants. As this test format uses 1/4 of the bacteria used in the plate incorporation assay, these spontaneous revertant numbers - except for TA100 - correlate well with those reported in the plate incorporation assay. The TA100 strain for the Ames MPF kits has been carefully selected and the conditions have been optimized to result in such a low number of revertants.

The Ames MPF and Ames II assays offered by Xenometrix have been designed to reduce the number of bacteria used and thus the required amount of test compound to a possible minimum while maintaining conditions to allow meaningful calculations of "fold induction". The inherent greater relative variability (standard deviation) of very low colony counts in the negative controls (spontaneous revertants) is taken into account by defining a "baseline" which includes this standard deviation. This baseline

is defined as "mean number of spontaneous revertants plus 1 standard deviation". The "fold induction" is then calculated relative to this baseline rather than - as in the plate incorporation test - relative to the mean number of revertant colonies.

Ours and other labs year-long experience with this calculation method has shown to be very realistic and has been corroborated by several comparative studies which have shown excellent concordances with the Ames plate incorporation assay comparable to the inter-lab variability of the classical Ames assay (Gee et al. (1998), Mut. Res. 412, 115-130; Flückiger-Isler et al. (2004), Mut. Res. 558, 181-197; Kamber et. al. (2009), Mutagenesis 24, 359-366),

A theoretically possible further reduction of the number of bacteria used could result in regular mean spontaneous reversion rates of 0 which would prohibit the calculation of a real "fold induction". Furthermore, the sensitivity of the test for weak mutagens could be reduced to the point that such compounds might not yield significantly elevated revertant numbers.

In conclusion, the Ames liquid microplate format by Xenometrix (Ames II, Ames MPF) is the optimal compromise between miniaturization and maintaining high enough counts for meaningful "fold induction" calculations.