



Automation of the AMES II-Assay: High-Throughput Screening of Mutagenic Substances

Background

A new version of the AMES Assay has been developed to identify base-repair substitution mutations upon detection of mutagens. Six *Salmonella typhimurium* strains have been constructed, each of which carries a different missense mutation in the histidine operon that is designed to revert uniquely to one of the six possible base substitutions. Reversion via the base substitution unique to each strain was verified by sequence analyses of more than 800 independent revertants induced by a variety of mutagens. AMES II permits identification of missense mutations caused by mutagens without the need to sequence by spectrophotometric analysis. AMES II strains can be combined and used as a single mixture for rapid screening due to minimal complementation among the 6 strains. Lower spontaneous reversion frequencies allow the detection of mutagens at lower concentrations without loss of sensitivity to a large range of doses. Liquid format in microtiter plates leads to increased sensitivity and easy automation.

The original Ames assay is a well established system in Aventis Pharma for mutagenicity testing during the development of compounds; the Ames II assay has been validated also in Aventis Pharma to combine the reliable experience of the original test with the high-throughput potential of the Ames II assay. Ames II mutagenicity Assays is available in suspension culture form with combined strains for HTS configuration. This kit is sold by Aniara (AMAX automated system). It can be used to replace or complement classical Ames test approaches. A workstation would provide the throughput needed.

Technical Requirements

- Expertise in AMES test analysis.
- An automated workstation with format versatility to provide 384-well configuration of plate formats.
- Test kits are on sale by Aniara.

Impact

- The assay detects the pointmutagenic endpoint of a substance which should be considered as a "red flag" for the mutagenic and carcinogenic properties of a compound.
- The objective for these assays is to rank about 100 compounds per week.
- The approach would help in the early selection of compound for progression in the critical path and impact the design of new compound libraries as well.
- The compound consumption for one test is only five milligrams.

Conclusion

The AMES II-Test makes it possible to make a rapid statement about the mutagenicity of a substance, using a very small amount of substance. Comparability of studies as against the standard Ames-Test lies around 90 %. The AMES II-Test is thus a practicable test system for the purposes of lead optimization.

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