The bacterial reverse mutation test

Commonly used acronym: *The Ames Test*
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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research, Regulatory use - Routine production
Type of method	In vitro - Ex vivo
This method makes use of	Other (e.g. bacteria): Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 & TA102 (alternatives for TA102 are E. Coli WP2 uvrA,or E. coli WP2 uvrA (pKM101))

DESCRIPTION

Method keywords

gene mutations
bacterial reverse gene mutation
OECD TG 471
histidine dependence

induced rat liver S9 fraction
standard plate incorporation assay
preincubation assay

Scientific area keywords

in vitro toxicology
genotoxicity
regulatory toxicology
pharmaceutical screening
carcinogenicity
mutagenicity
hazard assessment

Method description

The Ames test is a short-term bacterial reverse gene mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to permanent gene mutations. The test has been described in detail in OECD TG 471 and employs several histidine dependent Salmonella strains each carrying different mutations in various genes in the histidine operon. These mutations act as hot spots for mutagens that cause DNA damage via different mechanisms. When the Salmonella tester strains are grown on a minimal media agar plate containing a trace of histidine, only those bacteria that revert to histidine independence are able to form colonies. The number of spontaneously induced revertant colonies per plate is relatively constant. However, when a mutagen is added to the plate, the number of revertant colonies per plate is increased, usually in a dose-related manner. The standard plate incorporation test is the basic Ames test. Over the years modifications have been developed that enhanced the sensitivity of the test and allowed the testing of a wider range of chemicals, including gases and volatile chemicals. The best known modified Ames test is the preincubation assay.

Lab equipment

- Standard equipment for working with cell cultures;
- Colony Counter;
- Orbital shaking incubator.

Method status

Published in peer reviewed journal
Validated by an external party (e.g. OECD, EURL ECVAM,...)

PROS, CONS & FUTURE POTENTIAL

Advantages

- Short term, low cost & easy to perform;
- Simple, rapid and robust bacterial assay;
- Can detect suitable mutants in large population of bacteria with high sensitivity;
- Gold standard assay to predict human carcinogenic potential of a chemical by health regulatory authorities;
- First screen assay to determine the mutagenic potential of new chemicals and drugs;
- The specificity of the test strains can provide some useful information on the types of mutations that are induced by genotoxic agents;
- Detection of and distinction between direct acting mutagens and pro-mutagens that need metabolization.

Challenges

- Exogenous metabolic system is of animal origin (induced rat or hamster S9 liver fraction) and might be different from human metabolism;
- False positive results;
- The bacterial reverse mutation test may not be appropriate for the evaluation of

certain classes of chemicals, for example highly bactericidal compounds (e.g. certain antibiotics) and those which are thought (or known) to interfere specifically with the mammalian cell replication system (e.g. some topoisomerase inhibitors and some nucleoside analogues);
- Although many compounds that are positive in this test are mammalian carcinogens, the correlation is not absolute. It is dependent on chemical class and there are carcinogens that are not detected by this test because they act through other, non-genotoxic mechanisms or mechanisms absent in bacterial cells.
- Possible interference from biological samples eg plant extracts that contain amino acids (histidin) with the test system.

Modifications

Miniaturized Ames test like Ames MPF, Ames II, micro Ames test, mini Ames test...

Future & Other applications

Development of a exogenous metabolizing system that better mimics the human metabolism.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutat Res. 2000;455(1–2):29–60. doi: 10.1016/S0027-5107(00)00064-6. D. Maron, B.N. Ames, Revised methods for the Salmonella mutagenicity test, Mutat. Res. 113 (1983) 173–215. OECD Guidline 471 Bacterial Reverse Mutation Test.

Associated documents

Revised methods for the Salmonella mutagenicity test.pdf
OECD Guidline Bacterial Reverse Mutation Test.pdf

The Ames Salmonella microsome mutagenicity assay.pdf

Other remarks

Apart from the standard plate incorporation assay in our laboratory we perform also the Reductive metabolism assay (PRIVAL method) for AZO-compounds and the Modified Ames test for mineral oils.

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