



Project Summary

Compilation of Ames *Salmonella typhimurium* Plate Incorporation Test Protocols

Stephanie Toney and Larry D. Claxton

This compilation is meant to serve as 1) a reference for workers in the genetic toxicology field, 2) a starting point for creation of reference protocols for those who need precise Ames test protocols, and 3) a guide to understanding variations in test results. The full report, the result of an informal survey conducted by the U.S. Environmental Protection Agency, included laboratories that use the Ames test routinely. It is a simple compilation of the submitted protocols and cover letters. Any information not pertaining directly to the Ames test has been omitted and so noted on the cover sheet for each protocol.

The full report is not intended to make a comment on the competence or performance of any laboratory. It is important to remember that some laboratories may have Standard Operating Procedures that are more detailed than submitted protocols. Some laboratories use the protocol by Ames et al. as the main procedure and have sent only their modifications. However, the compilers do feel that this report is a useful reference. It can be a guide for writing better protocols, and it can provide a better understanding of the variations of results from different laboratories. A companion publication will also be available. It is a tabulated qualitative summary and comparison of submitted protocols.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research pro-

ject that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Bacterial tests for mutagenicity have been available for approximately 30 years. In 1951, Demerec et al. found that 19 out of 31 chemicals tested using an *Escherichia coli* reverse mutation system were mutagenic. After modifying the mutation system described by Demerec, Szybalski (1958) tested 431 substances for mutagenicity. Recognizing the inadequacies of the streptomycin resistance system, Ames (1971) published the methods for a bacterial system using a histidine-requiring mutant of *Salmonella typhimurium*. Malling (1971) was the first to merge a mammalian metabolizing system with a bacterial system in order to demonstrate the mutagenicity of dimethylnitrosamine.

In 1975, Ames et al. published a detailed protocol that incorporated the use of a mammalian metabolizing system thereby establishing the "Ames test" as a routine screening system for mutagenicity and potential carcinogenicity. Although the 1975 paper by Ames et al. provided a highly detailed protocol, many laboratories have introduced a variety of changes or additions. Also, summaries of international meetings have been published (Mattern and Greim, 1977; Seiler et al., 1979; de Serres and Shelby, 1979a; de Serres and Shelby, 1979b; Stich and San, 1979).

Since so many researchers and meetings have published suggested alterations and additions to the original protocol, the U.S. Environmental Protection Agency (EPA) decided to conduct an informal survey of laboratories that use the Ames test. A list of laboratories performing the Ames test in a routine manner was obtained from Dr. Mike Shelby at the National Institute of Environmental Health Sciences, Research Triangle Park, NC. An initial letter was sent to 46 laboratories included in this list. A follow-up letter was sent to the laboratories who had not responded within a one-month interval. As a result 33 laboratories responded to the request. The responses included 6 laboratories that no longer performed the Ames test, 1 that had closed, 23 that did perform the test and submitted protocols, and 3 that performed the test but elected to restrict the usage of their protocols. In addition, two EPA laboratory protocols are included within this compilation. A list of names and addresses of the laboratories that participated by sending protocols is found in Table 1.

Table 1. Names and Addresses of Participating Laboratories

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Ms. Melanie Baltezero
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Berkeley, CA 94710

This publication is a simple compilation of the protocols and cover letters submitted. Any submitted information not pertaining directly to the Ames *Salmonella typhimurium* plate incorporation test have been omitted; however, any omissions are indicated at the beginning of each protocol. The compilers and EPA understand that these protocols may not accurately reflect the competence or performance of any laboratory. Some laboratories may maintain Standard Operating Procedures (SOPs) that are more detailed than the submitted protocols, and some may have modified or expanded their procedures since submission of this document. However, the compilers do feel that many will find this report a useful reference, a guide for writing better protocols, and a guide to understanding why variations in results from various laboratories exist. A companion volume will be available which tabulates and discusses the similarities and differences between the submitted protocols.

Description of the Ames *Salmonella typhimurium* Plate Incorporation Test

The Ames test is a short-term bioassay for mutagenicity testing. The advantages of this test are the speed with which results can be obtained, usually three to four days, and the relative low cost. The Ames test is used for testing both pure

hemicals (cyclophosphamide and 6-mercaptopurine) and complex mixtures including mobile source emissions (diesel and gasoline) and comparative source emissions (coke oven, roofing tar, and cigarette smoke condensate).

The Ames test basically involves taking given sample and adding it to a strain of *Salmonella* in an agar overlay tube that is then plated on minimal media (see Figure 1). The sample is routinely tested at several dose levels with five Ames strains of *Salmonella*, with and without metabolic activation. The dose levels are plated usually in duplicate or triplicate. This plating involves approximately 120 plates/sample excluding the control plates.

After the bacteria, test compound, and possibly metabolic activation are added to the overlay tube, the contents are gently mixed and plated out on Vogel-Bonner minimal media plates. These plates are incubated for three days at 37 °C in the dark. After three days the plates are scored for mutants.

Five Ames strains are in widespread use—TA1537, TA1538 and its derivative TA98, and TA1535 and its derivative TA100. These strains are all histidine-dependent mutants and revert to wild type in the Ames test. Two of the strains detect frameshift mutations (TA1537 and TA1538), and one strain detects base-pair substitutions (TA1535). Strains TA98 and TA100 are less specific as to the type of mutants they detect. Strains TA98 and TA100 both contain an R factor plasmid that increases the sensitivity of these strains. All five strains have a *rfa* mutation creating a deficiency in the lipopolysaccharide cell wall therefore increasing permeability to macromolecules. The strains all have a *uvr B* mutation that decreases genetic reparability.

The mammalian metabolic activation system (S-9) is added to the overlay to identify compounds that require the metabolic activation mechanisms of mammals not found in bacteria. The mammalian metabolic activation system permits metabolites of the test compound to be tested for mutagenicity. Ames' activation system generally consists of a 9000 × g supernatant of Aroclor-1254-induced rat liver homogenate.

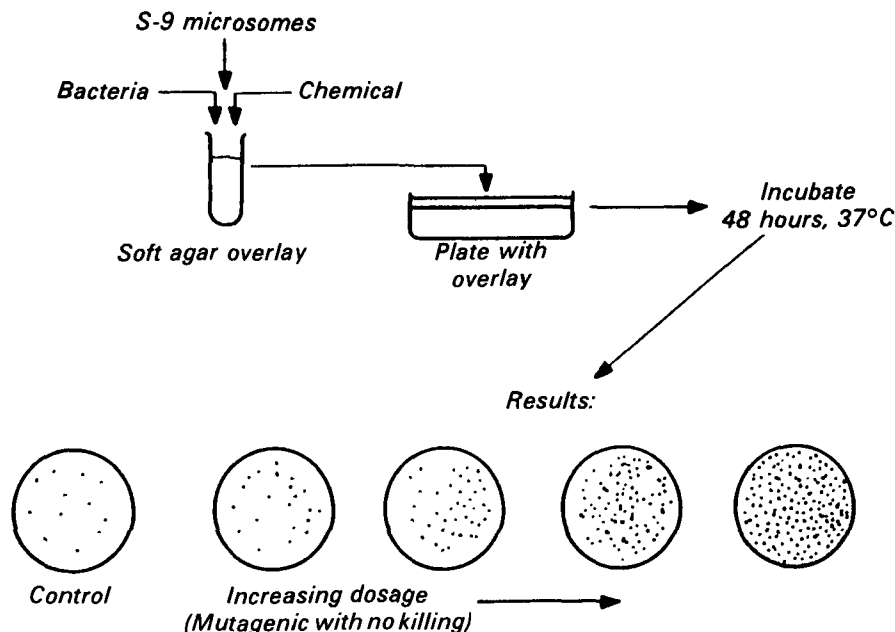


Figure 1. Schematic for plate incorporation test.

Stephanie Toney is with Northrop Services, Research Triangle Park, NC 27709; the EPA author Larry D. Claxton (also the EPA Project Officer, see below) is with the Health Effects Research Laboratory, Research Triangle Park, NC 27711.

The complete report, entitled "Compilation of Ames Salmonella typhimurium Plate Incorporation Test Protocols," (Order No. PB 83-113 290; Cost: \$37.00, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

The EPA Project Officer can be contacted at:
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