AMES TEST

I. OBJECTIVES

- To perform and interpret an assay to determine the mutagenicity of a compound.
- To discuss the specificity of selected mutagens.

II. INTRODUCTION

A simple assay was developed by Bruce Ames to test the mutagenicity of various chemicals. The test utilizes bacterial mutants containing specific transitions, transversions or frameshift mutations (small insertions or deletions) in genes of the histidine operon. Each of these types of mutations may be reversed by a specific complementary mutagenic event. The microbes are first grown in a rich broth containing ample histidine. The cells are then washed twice and inoculated onto a minimal agar plate. These cells, although auxotrophic for histidine, have accumulated enough of this amino acid to be able to go through 3-4 cell divisions without requiring exogenous histidine. A disk or drop of the test compound is placed in the center of the plate and the culture is incubated 48 hours at 37°C. A ring of distinct colonies around the disk indicates the effectiveness of the chemical in inducing a particular type of mutation.

Ames has demonstrated the usefulness of this assay system for a quick evaluation of the mutagenic properties of a chemical. It can be said that carcinogens are mutagens but not all mutagens are carcinogens. Ames test has been applied in many surveys of chemicals to define those substances that may present potential danger for humans who may be exposed to a specific compound.

The protocol is outlined below. The microbes are specific histidine-requiring mutants of Salmonella typhimurium derived in Dr. Ames' laboratory. These mutants are extremely effective in detecting classes of carcinogens that have little or no effect on the parent strains. The accuracy of the information derived from this test may be concluded from an experiment where 85% of the carcinogens tested (135/150) were detected as mutagens and less than 10% of 106 non-carcinogenic chemicals (based on animal studies) were positive in the Ames test.

For today's exercise, standard mutagen (ethyl methane sulfonate) will be provided; however, the group is encouraged to bring, in addition, one specimen from home, apartment, etc. to determine mutagenicity. This sample should be in liquid form and sterile (if possible). Many students like to bring as their test sample a detergent or cleaning agent that they regularly use at home and that is fine.
III. LABORATORY SUPPLIES

Ames agar plates 3/tablesde
Alcohol beaker, spreader & forceps 1 set/table
Vial containing mutagen (ethyl methane sulfonate) 1/lab
Sterile filter disks 1 jar/table
Transfer pipet as needed
Sterile Water 1 bottle/table
Culture of *Salmonella* (Ames strain; NR115), 1 ml 1/tablesde
Parafilm 1 roll/lab

IV. PROCEDURE (The students at each tablesde will work together as a group.)

First Session

1. Obtain 3 Ames plates and label them as EMS (mutagen), Unknown, and Control. Place 2-3 drops of the bacterial culture on each plate and spread well with a sterile spreader. Allow the bacteria to dry for a few minutes.

   **Important Note:** To sterilize the spreader, dip it in the 95% ethanol jar, shake the extra alcohol off by touching the inside of the jar above the alcohol level and then quickly take the spreader through a flame. Make sure that you do not hold the spreader in the flame for more than a second. It is the alcohol and not the heat that kills any bacteria present. Be extra careful as flaming alcohol drops may fall on objects and cause a fire hazard. Also while the alcohol is burning off, keep the spreader head down so the dripping alcohol will not spread to your fingers.

2. Using sterile forceps transfer a sterile filter disk to the center of each plate and tap gently to make sure the filter is attached to the surface of the agar.

3. Using a sterile dropper, place a drop of sterile water on the filter disk which is on the Control plate.

4. Repeat the above step with your unknown sample on the plate labeled Unknown.

5. Take the plate labeled EMS to the hood area. **Wear protective gloves when handling the mutagen!** Use a sterile dropper to add one drop of EMS to the filter disk. If your gloves become contaminated with the mutagen, remove them and place them in the biohazard bag in the hood. If your gloves are not contaminated, they can be placed in the regular trash.

6. Place plates face up (do not invert) at 37°C for 48 hours. Seal the EMS plate with a strip of parafilm. Your instructor will invert your plates later today, after making sure that the chemicals have diffused sufficiently into the agar.

Second Session
1. DO NOT OPEN THE EMS PLATE. Observe and record the growth and number of colonies on plates. Draw what you observe and label properly.

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Results of the Ames Test

NAME __________________ DATE __________ GROUP NAME ____________

PARTNER(S) ____________________________

Our Unknown test sample was:__________________________

1. Draw the appearance of the colonies in each of your plates below:

   Control  EMS

   Test sample
2. For each of your plates, report the number of colonies and interpret the results. What can you say about the genetic make up (his⁺ or his⁻) of the cells that grew on each plate or those that did not grow?

3. Was your test sample mutagenic?