Teacher Answer Key

AMES Assay CD ROM Activity

Use with the CD-ROM "Risky Business: Living in a Chemical World – Essentials of Cell Biology" developed at the University of Washington, Department of Environmental Health.

Materials developed by **Rebecca Milholland**, University of Arizona, Department of Pharmacology & Toxicology; and **Stefani Hines,** University of Arizona, Southwest Environmental Health Sciences Center

Objective: Teach students the importance of Bioassays for the determination of mutagenicity of a chemical and the importance of mutagenicity to cancer etiology.

Introduction

Today you will be learning about the AMES assay (one of the techniques scientists use to determine whether or not chemicals are able to mutate DNA) using a CD ROM as your guide. Open the folder on your desk top, click on the icon labeled Essentials of Cell Biology, and follow the directions from there. Use the CD ROM (Risky Business, Living in a Chemical World from the University of Washington, Department of Environmental Health) as a guide to fill in the Vocabulary definitions and answer the questions below.

Vocabulary - Each Vocabulary definition will be worth 1 pt.

1) Bioassay – *an experimental procedure that exposes organisms to a substance (e.g., soil, water, foreign chemicals) under controlled conditions to determine if the exposure affects the organisms*

2) Mutagen – *a chemical substance or physical agent (e.g. radiation) that can cause a mutation*

3) Carcinogenic – *capable of causing cancer*

4) Ames Test – *a test developed by Dr. Bruce Ames and used to determine the mutagenicity of chemicals. chemicals that are mutagenic to bacteria may be mutagenic and carcinogenic in humans*

5) Agar Petri Plate – *a plastic plate (or dish) containing a gelatin-like substance on which laboratory bacteria grow*

6) Gene – *a piece of DNA that controls cell structure and function. One gene makes one protein that has a specific function in the cell. There are over 100,000 different genes in human DNA. The DNA in every cell contains a copy of every gene*

7) Mutation – *a change in the DNA caused by chemical substances, physical agents (e.g. radiation), and biological agents (e.g. viruses)*

8) Incubate – *heating bacterial colonies, usually at 37 degrees C, so that they grow and reproduce*

9) Colony – *groups of bacterial cells on an agar petri plate grown from one bacterial cell that continuously divides*

10) Control plate – *an agar petri plate that duplicates test conditions in all respects except it does not contain the test substance*

11) Test plate – *an agar petri plate that contains the test substance and bacteria under specified conditions*

12) Dose Response Curve – *a graph that compares the response of organisms to varying doses of a chemical*

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Questions

1) What is the AMES assay? **1 pt.**

The AMES assay is a bioassay which uses mutations induced in bacteria by chemicals as a possible indicator of the ability of that chemical to produce mutations in humans.

2) The AMES assay helps determine whether or not a chemical could be carcinogenic. What does this mean and why is it important? **2 pt.**

A chemical that is carcinogenic is capable of causing cancer by inducing mutations in DNA. Mutations have been shown to be an important step in the cause of cancer.

3) What are the advantages to using the AMES assay? **2 pt.**

Fast
cheap way to identify mutagenic compounds
use bacteria instead of animals (save animals and have enough organisms to see an effect)

4) What other toxic effects (besides cancer) can occur following exposure to chemicals? **3 pt.**

Liver, kidney damage, and birth defects

5) What type of bacteria are used for the AMES assay? What disease can these bacteria cause? **2 pt.**

Salmonella typhimurium, foodborne illness called salmonellosis

6) How does the AMES assay measure mutation? In other words, what happens when the bacteria are exposed to a chemical that produces a lot of mutations? **1 pt.**

Chemicals which produce mutations enable the bacteria to the grow. (For more details, please see supplemental section below).

7) What steps are required to perform an AMES assay? Don't forget to include control and treatment plates (what are these?). **7 pt.**

a) special Salmonella typhimurium strain added to a previously prepared agar petri plate.
b) A well is cut in the center of the agar and a suspected chemical mutagen is added to the well
c) After a few minutes, the chemical diffuses away from the well to come into contact with the bacteria.
d) control plate – no chemical treatment
e) treatment plate – has chemical treatment
f) incubate bacteria at 37 degrees Celsius over for 2 days

g) count colonies on the control and treatment plates

8) What results (differences between control and treatment plates) would you expect to see if a chemical is highly mutagenic? **(2 pt. For answers, 1 pt. For correct labels)**
control plate –no or few colonies
treatment plate – many colonies

9) Why are there colonies on the control plate? **1 pt.**

There are some spontaneous mutations which allow the bacteria to grow.

10) What differences are there between the simulation shown in class today and an actual experiment? **2 pt.**

many petri dishes are used for a real experiment
different concentrations of chemicals used in an actual experiment

11) What is a dose response curve? Draw the dose response curve shown in this exercise (don't forget to label the axis). What does a Dose Response Curve for the AMES test tell a scientist about a chemical? **(3 pt. For graph - 2 for axis + 1 for graph, 2 pt. For other two questions = 5 pt. total)**

For graph see simulation.

A dose response curve is a graph of the number of colonies vs. dose of carcinogen. The DRC tells the scientist how effective different concentrations of the chemical are in inducing mutations in the bacteria.

Bonus Q: Why is there a sharp decline in the number of colonies at high doses of the chemical graphed in the exercise? **1 pt.**

At very high doses, the chemical is able to induce enough mutations to inhibit survival of even mutant bacteria.

Supplemental Questions

What is special about the *Salmonella typhimurium* strain used in the AMES assay? **(2 pt.)**

The bacteria are unable to produce histidine, an amino acid essential for growth. Thus, the bacteria are unable to grow in histidine free media.

What is the molecular mechanism behind the bacterial growth following mutation due to chemicals? **(2 pt.)**

Growth is allowed due to a reversion mutation in the gene for histidine production allowing the bacteria to produce their own histidine. Production of their own histidine allows the bacteria therefor to survive and replicate in histidine free media.

Student Sheet

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Vocabulary

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2) Mutagen –

3) Carcinogenic –

4) Ames Test –

5) Agar Petri Plate –

6) Gene –

7) Mutation –

8) Incubate –

9) Colony –

10) Control plate –

11) Test plate –

12) Dose Response Curve –

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Questions

1) What is the AMES assay?

2) The AMES assay helps determine whether or not a chemical could be carcinogenic. What does this mean and why is it important?

3) What are the advantages to using the AMES assay?

4) What other toxic effects (besides cancer) can occur following exposure to chemicals?

5) What type of bacteria are used for the AMES assay? What disease can these bacteria cause?

6) How does the AMES assay measure mutation? In other words, what happens when the bacteria are exposed to a chemical that produces a lot of mutations?

7) What steps are required to perform an AMES assay? Don't forget to include control and treatment plates (what are these?).

8) What results (differences between control and treatment plates) would you expect to see if a chemical is highly mutagenic?

9) Why are there colonies on the control plate?

10) What differences are there between the simulation shown in class today and an actual experiment?

11) What is a dose response curve? Draw the dose response curve shown in this exercise (don't forget to label the axis). What does a Dose Response Curve for the AMES test tell a scientist about a chemical?

Bonus Q: Why is there a sharp decline in the number of colonies at high doses of the chemical graphed in the exercise?