Mold Control

NIEHS OREGON STATE UNIVERSITY

INTRODUCTION

In this activity, your class will test three different cleaners and then will determine which cleaner is most effective for controlling mold. You will analyze a series of dilutions of one of the three cleaners and determine at what dilution the cleaner is no longer effective.

You will use everyday baking yeast for this experiment instead of mold. Yeast is very similar to mold. In fact, it is a single cellular fungus with the same properties as mold (also called fungus).

MATERIALS (PER GROUP):

- Baker's yeast solution (10^{-4} dilution)
- Disinfectant (Tilex, Lysol, or Borax)
- 8 sterile test tubes
- 10 ml sterile syringe
- 100 ml sterile water in beaker
- test tube rack or large beaker to hold test tubes
- 15 1ml sterile pipettes
- 8 sterile spoons
- 8 potato dextrose agar plates
- Permanent marker
- Gloves
- Safety goggles

A. EXPERIMENTAL SETUP

1. Obtain a test tube of yeast solution (10^{-4}) and a beaker of disinfectant from your teacher.

2. Be sure to wear gloves and safety goggles, especially when using the disinfectant.

3. Using the permanent marker, label your test tubes in the following way:

Test Tube	Label
1	100%
2	10%
3	1%
4	0.1%

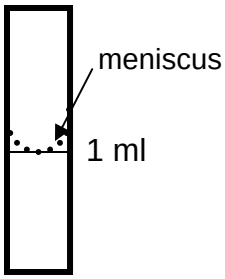
5	0.01%
6	0.001%
7	0.0001%
8	Control (0%)

4. You will need to label each of your 8 plates with the **Date, Group, Disinfectant, and Percent**. Do NOT open up the film yet. This will cause it to become contaminated. Using the permanent marker, write the following information on the **bottom** edge of the plates. The image to the right is an example.

5. Using your 10 ml syringe, add 9 ml of sterile water to all the tubes EXCEPT the one labeled 100%.

6. Using the same 10 ml syringe (be sure all the water is out), add 10 ml of the 100% disinfectant to the test tube labeled 100%.

7. **IMPORTANT:** Be sure that you use the pipettes properly and consistently between groups. It is very important that you use a new pipette for each different dilution. Mixing pipettes will result in contamination of solutions and a possible source of error in your results.



   a. Practice using a spare pipette with a small amount of water. Be sure that the bottom of the meniscus goes to the mark as in the figure to the right.

   b. Do not draw any liquid up into the bulb. It is very difficult to get out and will cause inaccurate measurements. Squeeze the bulb and **slowly** draw liquid up to the 1 ml mark on the narrow portion of the pipette.

8. Now make serial dilutions of the disinfectant**. Use a new pipette between each dilution**.

Check off each step as you go along so you don't lose track of where you are.

a. □ Place 1.0 ml of the 100% solution into a tube labeled 10%. Mix well.
b. □ Place 1.0 ml of the 10% solution into a tube labeled 1%. Mix well.

c. □ Place 1.0 ml of the 1% solution into a tube labeled 0.1%. Mix well.
d. □ Place 1.0 ml of the 0.1% solution into a tube labeled 0.01%. Mix well.
e. □ Place 1.0 ml of the 0.01% solution into a tube labeled 0.001%. Mix well.
f. □ Place 1.0 ml of the 0.001% solution into a tube labeled 0.0001%. Mix well.
g. □Remove 1 ml of the 0.001% solution and rinse it down the drain. (So all tubes have 9 ml)
h. **The last tube of water is the control. It has no disinfectant added.**

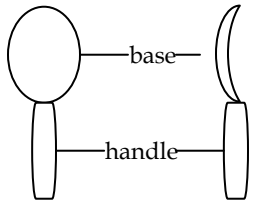
B. PERFORMING THE EXPERIMENT

1. Using a new pipette, add 1.0 ml of the yeast solution (10^{-4}) to each of the 7 solutions of disinfectant and to the control. Mix well. (The same pipette can be used to add the yeast to each solution.)

2. Leave all tubes at room temperature for 10 minutes.

3. Now you are ready to "plate out" the solutions onto the agar plates.

   a. Gently shake each test tube before you remove liquid to be sure it is well mixed.
   b. **Using a new pipette**, draw up about 0.5 ml of the solution from the test tube labeled 100%.
   c. Lift the lid covering the plate.
   d. Hold the pipette 1 inch above the surface of the plate and squeeze the bulb very gently and slowly letting one drop fall about every 1 second. SLOWLY put **4 drops** of the solution onto the center of the plate. Do not scatter the liquid over edges of the plate.
   e. Remove a sterile spoon from the water by the handle without touching the base of the spoon with your skin.



   f. Using the round backside of the spoon base GENTLY spread the liquid over the entire surface of the plate as shown in the picture below.

g. For 15 seconds spread the liquid over the entire surface of the agar by continuously moving the spoon over the plate in a uniform motion.
h. When the liquid on the plate has been completely spread, replace the lid and set the plate aside before moving onto the next solution.

4. Repeat this procedure with each solution and the control. **Use a new pipette for each solution. Be sure to gently mix the tube just before taking out the solution to be sure you get a good sample.**

5. When you are finished with each solution, collect your plates and give them to your teacher. Your teacher will put these in a warm place for the yeast to grow, this will take about 48 hours. The plates should be incubated upside down so condensation does not drip onto the surface of the agar.

C. COLLECTING DATA

1. After the yeast have formed colonies, count the number of shiny, circular white colonies.

   a. If your plate has hundreds of colonies, it will not be easy to count. If the colonies are evenly spread out over the entire plate, you can draw a line on the outside of the plate to divide the circle in half, or divide it into four quarters. If you count half of the colonies, multiply your count by two. If you count one-quarter, multiply your count by four.
   b. If your plate has discolored spots, mold, or seems abnormal, consult your teacher.

2. Record your data on the **Mold Control Experiment Worksheet**. Calculate the difference between the yeast in the disinfectant solution and the control.

3. For the right column of the table, subtract the number of colonies you counted for each dilution from the number that were in the control. If you end up with a negative number, write "0" in the right column.

4. Plot the numbers you calculated in the right column on the Dose-Response Curve. Label the **LOEL**, **LD100**, and **LD50** on your Dose-Response Curve.

5. Share your results with the rest of the class.

6. Complete the **Summary Questions**.

Name_____ Disinfectant_____

MOLD CONTROL EXPERIMENT WORKSHEET

INSTRUCTIONS:

1. Enter the number of yeast colonies that grew on your control treatment in the first table. This variable is called "C"

2. Enter the number of yeast colonies that grew on the petrifilms for different disinfectant concentrations.

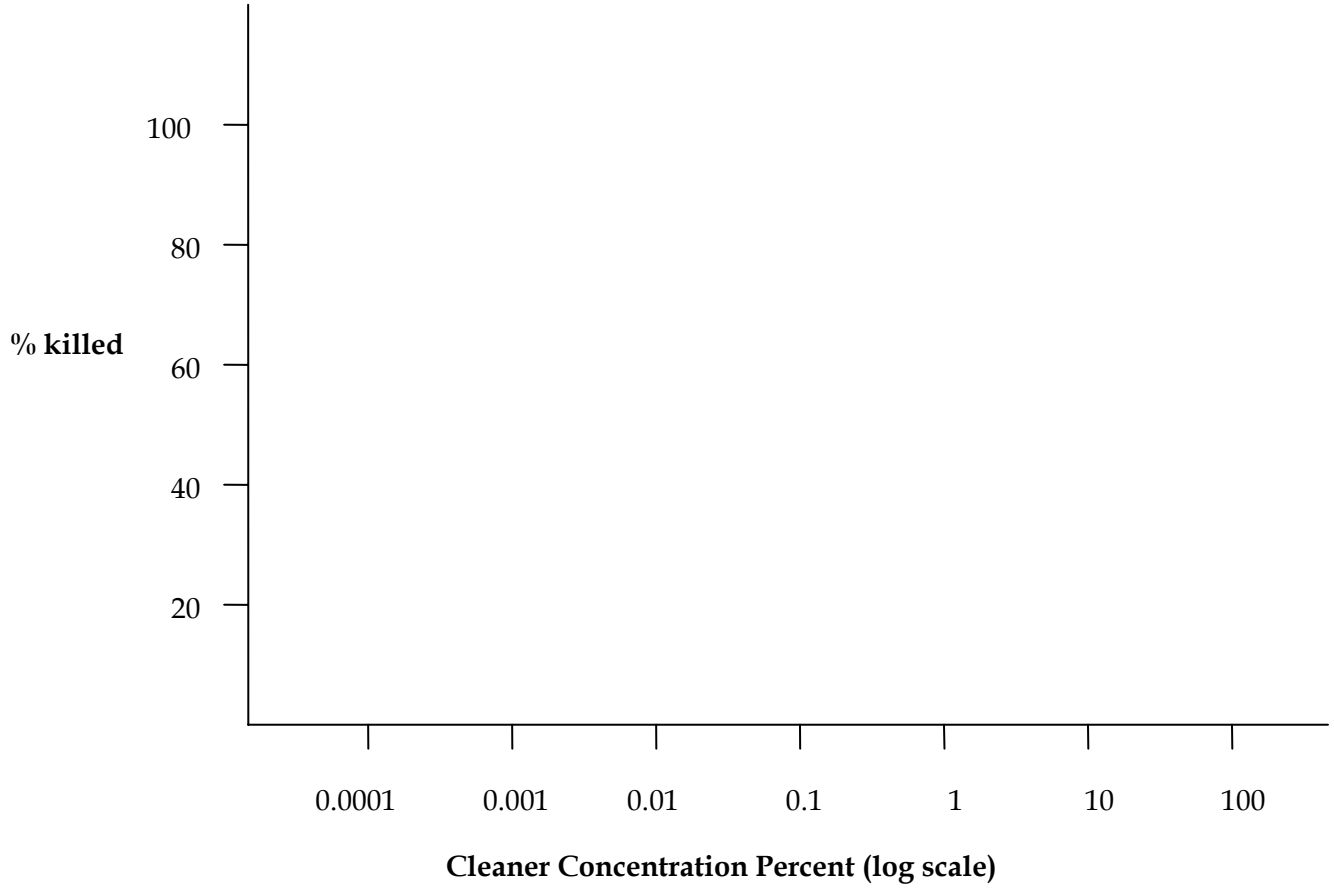
3. Subtract the number of colonies that grew on the treatment films from the number of colonies that grew on the control film. This will determine the number of colonies killed by the disinfectant.

4. Plot the Percent killed vs. Cleaner Concentration on the Dose-response curve on the graph below.

	Number of yeast colonies
Control	**C=**

Cleaner Concentration	**Number of yeast colonies (T)**	**Percent killed** $\frac{C-T}{C} \times 100$
100%		
10%		
1%		
0.1%		
0.01%		
0.001%		
0.0001%		

DOSE-RESPONSE CURVE FOR _____



5. The threshold of effectiveness is the point of the curve at which you begin to observe an effect. In toxicology, this point is known as the **LOEL** (Lowest Observable Effect Level). Mark this on your dose-response curve.
6. There is also a point at which the effect does not increase, despite increasing dose and all of the test population is killed or 100% death. This is called the **LD100** (lethal dose 100%). Mark this on your dose-response curve. (Mark the lowest dose that kills 100% of the yeast colonies.)
7. Toxicologists use the **LD50** value to compare the toxicities of different chemicals. This is the dose at which 50% of the test population is killed. Mark the LD50 on your dose-response curve.

A chemical with a lower LD50 value is more toxic than a chemical with a higher LD50. Why? It takes less to cause the same toxic effect.

Example: Chemical A has an LD50 of 45. Chemical B has an LD50 of 10. Which is more toxic? (answer on next page)

NAME_____ **CLEANER**_____

SUMMARY QUESTIONS

1. Why did you use a control (water) treatment?

2. Which cleaner was the most effective? How do you know?

3. Was there a concentration below which the cleaners were no longer effective in controlling the yeast?

4. Was there a concentration above which the cleaner did not increase control of yeast?

Answer: Chemical B is more toxic.