

Mold Control



OREGON STATE
UNIVERSITY

DESCRIPTION:

Using three types of cleaning solutions (sodium hypochlorite, quaternary ammonium compounds, and borates), students analyze which product is most effective for controlling yeast (substitute for mold). A dose-response curve is developed to analyze the toxicity of cleaning products to yeast.

RATIONALE:

In the Hydroville Indoor Air Quality Scenario, students will determine how to control mold problem in Hydroville Middle School. This activity gives them the opportunity to test several potential cleaning products

PURPOSE/GOALS:

Students will be able to:

- Develop a dose-response curve.
- Understand the effect disinfectants have on yeast growth.
- Understand the purpose of using an experimental control.

TIME ESTIMATE:

Prep: 60-90 minutes

Activity: 50-55 minutes

MATERIALS (COMPLETE LIST):

- Baker's yeast (Fleishmann's Rapid Rise - 0.25 oz packet)
- 35 sterile test tubes w/caps or Parafilm
- 3 test tube racks or large beaker or cups
- Tilex Mildew Remover (EPA Reg No. 5813-24)
- Lysol Antibacterial Kitchen Cleaner (EPA Reg. No. 777-66)
- Borax cleaner solution (20 Mule Team works well -10 g in 250 ml warm water)
- 500 mL sterile water
- 6 sterile beakers (100 or 250 ml)
- 24 yeast and mold (Y&M) 3M petrifilms
- 3 Y&M 3M petrifilm spreaders
- 5 10 mL sterile syringes
- 55 1 mL sterile syringes or pipettes (disposable ok)
- 3 small beakers (need to hold 100 mls)
- 3 sandwich size Ziploc bags
- Scale or balance
- hot plate or autoclave if available
- 3 permanent markers (ex. Sharpie)
- Gloves (one pair per student)
- Safety goggles (one pair per student)

MATERIALS (PER GROUP OF STUDENTS – DIVIDE CLASS INTO THREE GROUPS):

- Baker's yeast solution (10^{-5} dilution)
- 15 ml disinfectant (Tilex, Lysol, or Borax) in sterile beaker
- 8 Y&M petri films
- Y&M spreader
- 8 test tubes plus caps or Parafilm
- 1 10 ml sterile syringe
- 100 ml sterile water in beaker
- Test tube rack or large beaker to hold test tubes
- 15 1ml sterile syringes or pipettes
- Permanent marker
- 1 sandwich size Ziploc bag
- Gloves (one pair per student)
- Safety goggles (one pair per student)

MATERIALS TO PHOTOCOPY:

Transparency 1: Growth of Yeast Colonies After Incubation

Transparency 2: Making 10-fold Serial Dilutions

Student instructions and worksheets

BACKGROUND INFORMATION: ** Information summarized from: Trautmann, N. et al. 2001. *Assessing Toxic Risk*. National Science Teachers Association Press. Arlington, VA.

Why conduct toxicology experiments?

“One of the reasons for studying toxicology at the high school level is its relevance to everyday life. On a daily basis we are confronted with news reports about toxic chemicals in our food, water, and environment. How do we decide which of these are worth worrying about? Each of us must make individual decisions about questions such as, “Should I buy bottled water, or is it safe to drink water from the tap?” We also can exert political pressure to influence broader societal questions such as, “Should the federal government ban sales of saccharin?” or “Should the town spray herbicides to control weed growth on the highways?” Too often these decisions are based on misconceptions about what is “safe” and what involves too great a risk. In learning the basic concepts of toxicology, students will become better prepared to make reasoned decisions about issues such as these.”

Excerpted from: Trautmann, N. et al. 2001. Assessing Toxic Risk. National Science Teachers Association Press. Arlington, VA.

Toxicology can:

- Connect classroom science to relevant issues in local communities;
- Provide a link between biology, chemistry, environmental science, ethics, law, and human health;
- Highlight the interplay between science, ethics and human values in the development of public policy;
- Provide authentic open-ended scientific investigations for students;

- Help develop students' critical thinking skills (e.g. how to interpret and explain experimental results and relate these to issues dealing with human health and the environments;
- Develop students' understanding of chemical risks.

What is the definition of dose and response?

Dose: **The measured amount that enters a body.** Often this is reported as the concentration of the chemical of concern per unit of body mass. Example: A dose of 20 mg/kg means that there is a concentration of 20 mg of chemical per kg of body weight. In a 165 lb person (75 kg), a dose of 20 mg/kg means that 1500 mg have entered the body (75 kg x 20 mg/kg = 1500 mg).

Response: **The effect of the chemical on the body.** This can be negative, in the case of a harmful chemical, or positive, such as therapeutic drugs. However, in a large enough dose, even a beneficial substance can have negative effects.

What do dose-response experiments tell us?

In a dose-response experiment, a population of test organisms is exposed to increasing doses of a single chemical and the range of responses of the test organism to the chemical is recorded. In dose-response experiments, the composition and concentrations of chemical solutions are known. Often in dose-response experiments, a chemical that is harmful at high doses can sometimes have no effect or even beneficial effect at low doses (ex. therapeutic drugs.) Overall, dose-response experiments demonstrate the toxicological principal that **"the dose makes the poison."** The resulting data can also be used to determine the LD₅₀ (the dose of the test chemical that is lethal to 50% of the bioassay organisms) or other measures of the response to chemical solutions. These experiments can also determine the Lowest Observable Effect Level (LOEL), or the dose at which the researcher begins to see an effect.

LD₅₀/EC₅₀ Experiments: **LD₅₀** (lethal dose 50%) experiments measure the dose of a test chemical which will kill 50% of the exposed organisms within a specific time period. LD₅₀ is an extremely useful reference because it allows toxicologists to compare and rank the toxicity of different chemicals to one another. However, lethality is not the only effect that toxicologists measure, and in fact, dose-response experiments can also measure the concentration at which therapeutic effects occur (**EC₅₀**, Effective Concentration 50%) or the dose at which negative but non-lethal effects occur (**TD₅₀**, Toxic Dose 50%). *LD₅₀ is an extremely useful reference because it allows toxicologists to compare and rank the toxicity of different chemicals to one another.*

TD₅₀ Experiments: Dose-response experiments can also be conducted to determine the concentration of a test chemical that causes a specific negative effect (e.g. inhibition of growth) other than death. TD₅₀ (Toxic Dose 50%) is the concentration at which 50% of test organisms that display the specific negative response.

Bioassays: Laboratory mice vs. Other Organisms in Toxicology Experiments

Dose-response bioassays provide information about the acute toxicity of a single chemical to the bioassay organism from short-term, acute exposures. Dose-response experiments can demonstrate the range of effects of chemicals on bioassay organisms, from no observable effect to very high toxicity. Since other types of organisms might respond differently to the same chemical, it is a good idea to try bioassays using several different species.

When scientists want to determine the possible impacts of a substance on human health, they often conduct dose-response bioassays using rats, mice, or other laboratory animals. Laboratory mice are used for bioassays related to human health because they provide a reasonable model of human response to chemicals. The results of these experiments are often LD₅₀ values (see above). Scientists use these bioassay results to compare the toxicity of various compounds and then to predict the potential effects of these same chemical on human health.

Bioassays are also used to test the toxicity of environmental samples, and in these types of bioassays we are interested in the response of organisms other than humans. In environmental sampling bioassays can indicate the toxicity of an unknown solution or environmental sample. In these cases instead of using laboratory rats or mice, it makes sense to conduct bioassays with organisms that are typical of the environment being tested. Herbaceous/aquatic plants, aquatic invertebrates, fish, worms, single-celled algae and fungi are all useful for bioassays because they are representative of the types of organisms found in aquatic or terrestrial ecosystems, and they are responsive to many types of environmental contaminants. Dose-response experiments provide a useful frame of reference for bioassays because these experiments test mixtures of unknown chemicals found in environmental samples and can be compared to laboratory run dose-response experiments as a reference of response for specific doses of known chemicals.

How do scientists choose what types of bioassay organisms to use in their experiments? The species used for bioassays should be sensitive to various types of chemicals and chemical mixtures. The bioassay organism should also be relatively easy to keep alive in the laboratory. No single species provides the perfect bioassay. Each responds in its own way, so toxicity testing usually includes more than one species in order to provide a more complete picture of toxicity. The species of choice may also depend on the type of chemicals being studied and the purpose of the experiments. For example, suppose that you want to investigate the impact of household cleaners advertised to be effective in killing molds on non-porous hard surfaces. Using bioassays with yeast or other fungi could help determine whether a specific household chemical is toxic to molds and at what concentrations.

Why Yeast Bioassays?

In the Mold Control toxicology activity, common baker's yeast is used as the test organism instead of mold. This activity uses yeast as the bioassay organisms for several reasons: (1) Yeast is very inexpensive and easily found in most grocery stores, is activated easily in water, and is good for use in serial dilutions; (2) Yeast grows rapidly and develops distinct round colonies that are easy to count; (3) Yeast are unicellular fungi and are closely related to the molds commonly found in and around homes; (4) Yeast is less allergenic than molds (molds can aggravate allergies in some students). Because the purpose of this activity is to determine which cleaner will control mold, yeast is a good representative model organism for fungi.

TERMINOLOGY:

Yeast	Dose-response curve
Fungus/fungi	Toxicity
Control Treatment	Serial dilution
LOEL (lowest observable effect level)	LD50 (lethal dose 50%)

SUGGESTED LESSON PLAN:*Getting Started*

1. Sterilizing materials (can be done ahead of time)
 - a. Water should be boiled for 30 minutes or autoclaved for 20 minutes
 - b. Test tubes and small beakers should be boiled for 30 minutes (separate from the sterile water) or autoclaved for 20 minutes. You can also bake the dry glassware with foil on the tops in the oven for 1-2 hours at 250° F. It is not advised that you also prepare food while doing this.
 - c. If you boiled the glassware, you can place clean aluminum foil over the tops of the test tubes and beakers to keep them clean.
2. Pour out about 20 ml of the disinfectant into 3 separate sterile beakers for the students. Label the beakers. Divide the students into three groups, one for each disinfectant.

IMPORTANT SAFETY INFORMATION: The Tilex and Lysol solutions should not be mixed. This can result in toxic gases. Classroom should be well ventilated if possible.

Lysol is irritating to the eyes. Tilex is irritating to the skin and eyes. Tilex contains sodium hypochlorite (bleach) which may damage clothes. Borax can also irritate eyes. Students should wear gloves and goggles while they are working with the disinfectants.

3. Preparation of yeast solutions can be done about 20 minutes before activity but not more than 1 hour or yeast will become inactive. You can do this while the students are making their own serial dilutions. It will take about 10 minutes to prepare.

You will need:

- Baker's yeast
 - Scale or balance
 - Sterile 10 ml syringe or graduated cylinder
 - 8 sterile test tubes and caps or Parafilm
 - 100 ml sterile water in sterile container (beaker or cup)
- a. Review the overhead transparency: **Making 10-fold Serial Dilutions**
 - b. Label 8 test tubes in the following way:

Test Tube	Label
1	1
2	10 ⁻¹
3	10 ⁻²
4	10 ⁻³
5	10 ⁻⁴
6	10 ⁻⁵
7	10 ⁻⁵
8	10 ⁻⁵

- c. Using a sterile 10 ml syringe, add 9 ml sterile water to each test tube.
- d. Weigh out 1 g of yeast. Add this to the tube labeled "1." Mix well by capping or covering with parafilm, invert several times. You can also gently tap the bottom of the test tube against the palm of your hand to swirl the solution. Wait several minutes. Mix again until there are no clumps. **It is very important that this starting solution is well mixed.**
- e. **Yeast Serial Dilution Steps (check boxes to help you keep track)**
- Place 1.0 ml of the solution 1 into a tube labeled 10^{-1} . Mix well.
 - Place 1.0 ml of the solution 10^{-1} into a tube labeled 10^{-2} . Mix well.
 - Place 1.0 ml of the solution 10^{-2} into a tube labeled 10^{-3} . Mix well.
 - Place 1.0 ml of the solution 10^{-3} into a tube labeled 10^{-4} . Mix well.
 - Place 1.0 ml of the solution 10^{-4} into a tube labeled 10^{-5} . Mix well.
 - Place 1.0 ml of the solution 10^{-4} into a tube labeled 10^{-5} . Mix well.
 - Place 1.0 ml of the solution 10^{-4} into a tube labeled 10^{-5} . Mix well.
- Mix the solution gently just before you draw liquid from it to ensure that it is well mixed. Using a sterile 1 ml pipette, transfer 1 ml from solution 1 to the test tube labeled 10^{-1} . Cap and mix well. **Use a NEW pipette for each dilution.**
- f. **You will create THREE test tubes of 10^{-5} .** Transfer 1 ml from the 10^{-4} solution to the tube labeled 10^{-4} . Do this step two more times. (You can use the same pipette.) Students will use the 10^{-4} dilution for their experiment.
- g. The yeast solutions can be rinsed down the drain and the glassware can be cleaned with soap and water.
3. Review the 3M Yeast and Mold Petrifilm Interpretation Guide or watch the video clips on the 3M website. They are very short and useful to see how the petrifilms should be used. You will need Quicktime to view these.

http://www.3m.com/microbiology/home/products/petrifilm/pp_vid.html

These two videos are the most useful:

Three Simple Steps

Plating Tips for Petrifilm Plates without a Foam Dam

If you do not have access to these videos, these tips will help students use the petrifilms.

- Lift the bottom edge of plastic film.
- Do not touch** the inside of the plastic film or the grid surface. This will introduce contamination.
- Slowly place 1 ml of liquid in the center of the grid as one large drop (do not scatter over surface)
- Allow the plastic film to fall down onto the liquid.
- Using the spreader, press firmly for about 5 seconds to disperse the fluid.
- Do not twist or move the spreader while pressing.

Doing the Activity

1. Discuss the concept of sterility with the students. Encourage them to not touch anything but the handles of the syringe and the bulbs of the pipettes. Students should not touch the inside of the petrifilms. They should only handle the bottom edge of the plastic film.
2. To stage this activity, show students *Transparency 1: Dilution scheme*. Discuss with students the purpose of preparing diluted disinfectant solutions. From this technique the concentration of disinfectant that effectively kills yeast will be determined.
3. If possible, show students the 3M video for petrifilms. If not possible, discuss proper procedure for using petrifilms.
4. Each group of students will add 1 ml of their 10^{-5} yeast solution to their serial dilutions of disinfectant. They will mix well and then place 1 ml of each disinfectant solution onto a petrifilm.
5. The petrifilms take between 3-5 days to incubate depending on the temperature. In this time, the yeast will form colonies. They will appear as blue dots on the films. **If the colonies develop and begin growing into one another before it is time for the students to count the colonies, place the films in the refrigerator to stop the yeast growth.**
6. After incubation, have students count the blue colonies that formed on their films. Students should record their results in the data table and then plot the results on the dose-response curve.
7. Troubleshooting:
 - a. If there are films which have a blue ring around the edge, this means that there were too many yeast cells.
 - b. If there are no colonies but there is a bluish tinge in the circle, this also means that there are too many colonies.
 - c. If there are spots other than small, defined blue circles, instruct students not to include these in their counts. This is probably contamination.
 - d. If there are a very large number of colonies, students can count half or one-quarter of the colonies (draw lines on the plastic surface of the film) and then calculate an estimate of the total number of colonies on the film.

If something should go wrong and the students cannot obtain data from their petrifilms, you can give them the data at the end of the student section. Do NOT provide this data unless the experiment does not work. These are just a backup plan.

⇒ Plotting Dose-Response Curve:

Students will do calculations on their student worksheet. They will then plot the results for their disinfectant on the dose-response curve. The x-axis is the % concentration of cleaner. The y-axis is the % yeast colonies killed. Answers will vary for the different cleaners.

SUMMARY QUESTIONS (TEACHER KEY)

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

In order to test the effectiveness of a treatment, it is important to compare it to a sample that does not receive any experimental treatment. In this case, the control is only in water. Control samples go through all the same procedures as the rest of the experimental samples except for the fact that they do not receive a treatment.

⇒ Which cleaner was the most effective? How do you know?

Answers may vary. Tilex is typically the most effective. Borax results may be similar or slightly less effect than Lysol.

Results should show a decrease in yeast colonies as concentration of disinfectant increases. At high disinfectant levels, there will be no colonies. The lowest concentrations may show no effect.

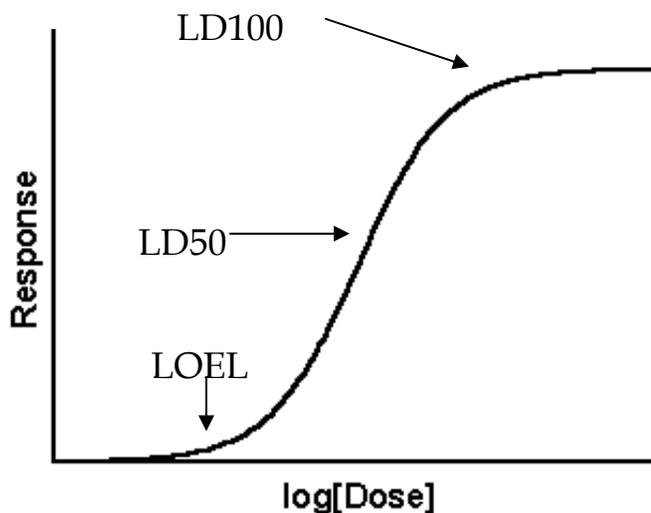
3. Was there a concentration below which the cleaners were no longer effective in controlling the yeast?

Answers will vary. This will be just below the LOEL. In toxicology, this is called the NOEL, no observable effect level.

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

This is usually the LD100, the point at the top of the curve where it begins to flatten. However, if the cleaner does not kill all yeast colonies, then the LD100 is higher than concentrations tested in this experiment.

Dose Response Curves typically have the general shape shown in the graph below. The students may not have enough data points to complete the entire dose response curve. However there should be a level at which there is no effect and there should be a level at which the effect no longer increases at concentration increases. The LD50 is the dose at which 50% of the test population dies.



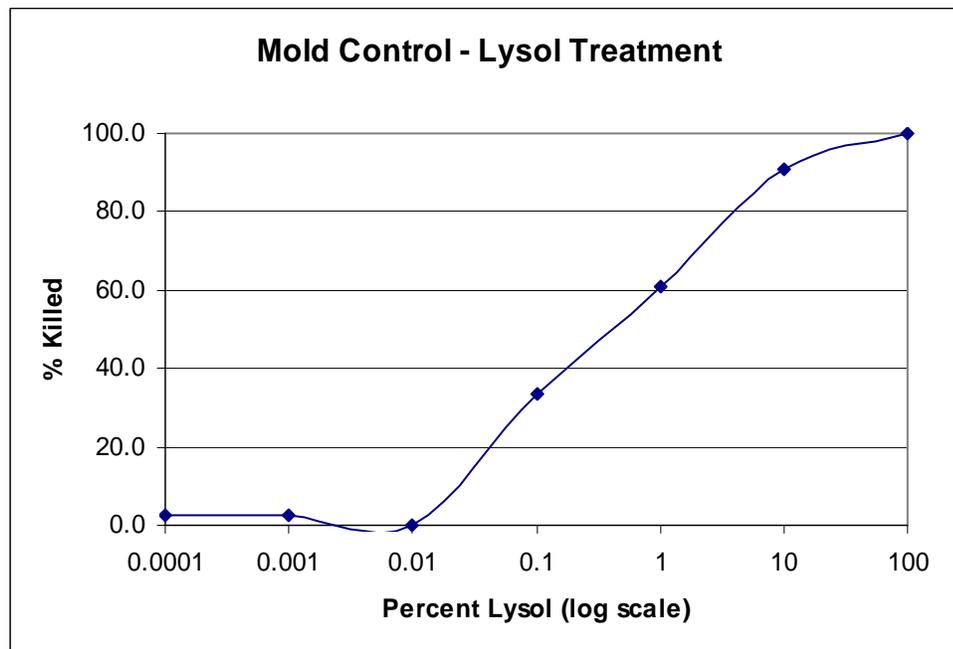
SOLUTIONS FOR BACKUP DATA

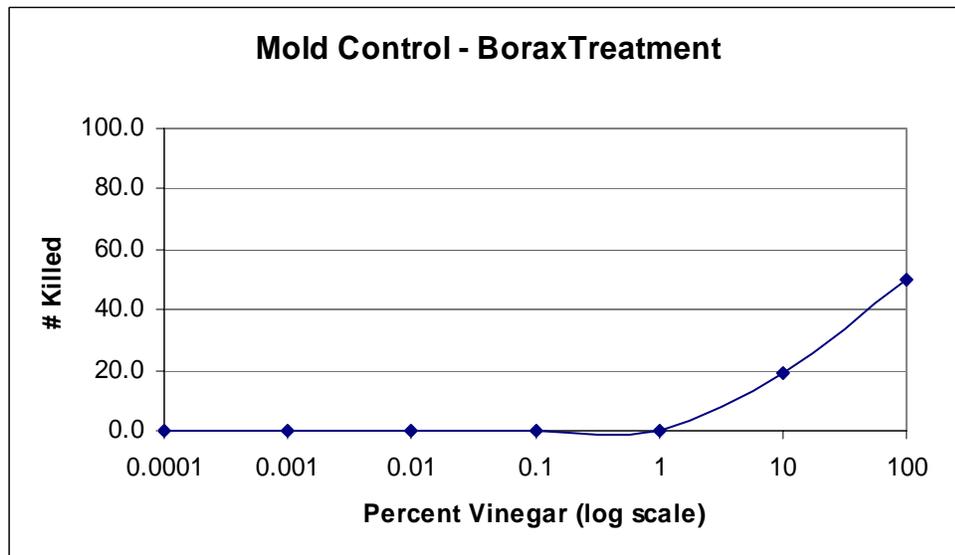
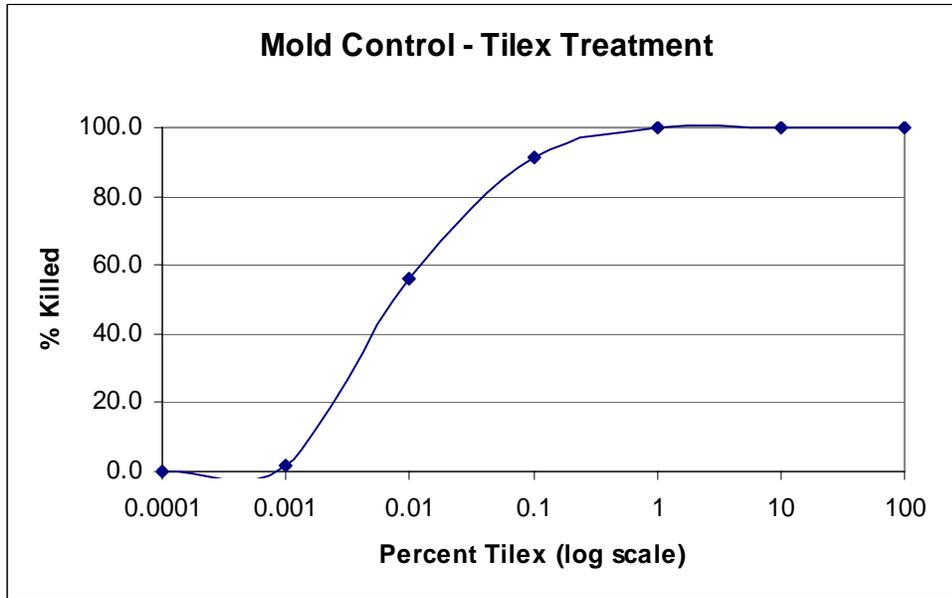
Raw Data

	Lysol	Tilex	Borax
Control	131	102	126
0.00%	128	109	130
0.00%	128	100	137
0.01%	136	45	135
0.10%	87	9	143
1%	51	0	147
10%	12	0	102
100%	0	0	63

Percent killed (control – treatment)/control adjusted – neg. values are zero

	Lysol	Tilex	Borax
0.00%	2.3	0.0	0.0
0.00%	2.3	2.0	0.0
0.01%	0.0	55.9	0.0
0.10%	33.6	91.2	0.0
1%	61.1	100.0	0.0
10%	90.8	100.0	19.0
100%	100.0	100.0	50.0





INFORMATION RESOURCES:

Toxicology Tutorials from the National Institute of Health. Very user-friendly and very informational

<http://sis.nlm.nih.gov/Tox/ToxTutor.html>

Dr. Fungus; a fun, educational website for all things fungi

<http://www.doctorfungus.org/>

Yeast (Budding, Fission, and Candida)

<http://genome-www.stanford.edu/Saccharomyces/VL-yeast.html>

Red Star Yeast Science pages; other great science projects with yeast

<http://www.redstaryeast.net/science.htm>

MATERIALS RESOURCES:

3M Yeast and Mold Petrifilms – 3M Microbiology – 1-800-328-1671 (\$84.40 for a box of 100 films)

Carolina Biological Supply – 1-800-227-1150 (\$57.95 for 50 films)

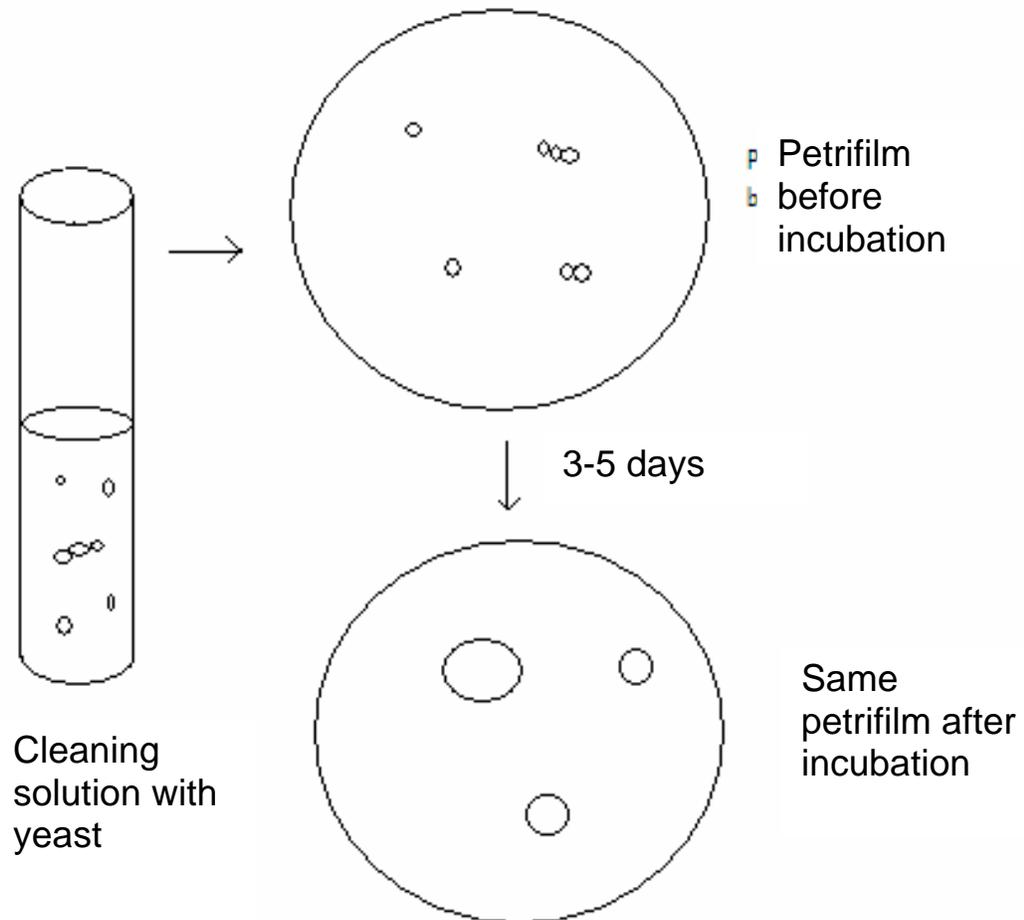
***If you have a contact at a college or university in a science department, they may be able to order a **free** box of 100 films from 3M through the 3M University Program.

Visit <http://www.3m.com/microbiology/eduhome/univ.html>

Sterile Graduated Disposable Pipettes – Fisher Scientific 1-800-766-7000 or www.fishersci.com (\$56.25 for box of 500 individually wrapped)

*Prices effective as of October 1, 2003

GROWTH OF YEAST COLONIES AFTER INCUBATION



MAKING 10-FOLD SERIAL DILUTIONS

