Investigations into Chemical and Physical Control of Microorganisms

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Authors

Suzanne Anglehart
University of Wisconsin – La Crosse
La Crosse, Wisconsin
Email: anglehar.suza@uwlax.edu

Annie Voyles
Central High School
La Crosse, Wisconsin

Bonnie Jo Bratina
University of Wisconsin – La Crosse
LaCrosse, Wisconsin
Email: bratina.boni@uwlax.edu

Abstract

This is a two-part exercise on chemical and physical control of microbes. In Part I normal skin flora organisms will be isolated and used in a disc diffusion assay to test the efficacy of various household products and antibiotics. Lab skills mastered in Part I will be employed in Part II, an investigative exercise where factors affecting the effect of UV exposure on *Serratia marcescens* will be determined through group-designed procedures. Experimental set-ups will be exposed to a UV lamp in a biosafety hood. Growth after incubation at room temperature will be recorded and compared to results from other groups.

Activity

INTRODUCTION

Learning Objectives.
At the completion of this exercise students will be able to appreciate the diversity of microbes that make up normal flora, conduct a disc diffusion assay to test for antimicrobial activity, and design and carry out an experiment to demonstrate the effect of UV radiation on a bacterial culture. Students will increase their skill levels in analysis and communication through interpretation of data and presentation of results in oral and written forms.

Background.
In our lab, this is the second in a series of three investigative exercises. Our first investigative exercise taught the scientific method. Therefore, our students are versed in designing a controlled experiment. If this is your first investigative experiment, you will need extra time to discuss the scientific method prior to Part II. No further techniques are required as Part I of this exercise introduces the techniques needed for Part II.

PROCEDURE

Materials.

Part I.

- 2 tryptic soy agar (or any general purpose media) plates per person
- Sterile filter paper discs (1/4-inch circles)
- Marking pens
- Forceps
- Microfuge tubes with 0.5 ml of sterile saline, phosphate-buffered saline, or phosphate buffer (for resuspending bacterial cells)
- Laboratory burners
- Alcohol jars
- Sterile toothpicks
- Sterile swabs
- Metric ruler
- Assorted antiseptics, disinfectants, antibiotics, soaps, and cleansers (this includes an item or items students bring from home to test)
- 37^{o}C incubator

Part II.

- UV radiation source

- Sterile swabs
- Tryptic soy agar plates (2 or 3 per group)
- Culture of *Serratia marcescens*
- Tube rack
- Plastic wrap
- Cardboard or tinfoil

Student Version.

Part I. Chemical control

Wash your hands! This is something that we all learn at a very early age is important for good hygiene. It is hard to believe that, back before microbes were proved to cause infectious disease, this was not common practice. In 1847 Ignaz Semmelweiss, a Hungarian physician, was concerned with the high number of women dying of childbed fever contracted after giving birth. He observed doctors going from one birth to the next or even from postmortem dissections to maternity without washing their hands. He instituted a policy of hand washing in a dilute chlorine solution just prior to delivery, and fever incidence dropped from 50% to 1%. Although this seems convincing, he received vicious criticism from the medical community, who ridiculed the hand washing procedures. The abuse took a heavy emotional toll on Semmelweiss, and he was committed to an asylum where, ironically, he died of an infection caused by the same organism that causes childbed fever. His work, however, inspired Joseph Lister whose tenaciousness and aggressive promotion finally overcame opposition to sanitation practices. So, think of Semmelweiss and Lister next time your mom yells, "Wash your hands!"

It is necessary to control microbial growth and survival to prevent disease in man, to prevent disease in plants and animals of economic importance, and also to prevent spoilage of commodities. In order to achieve this, a variety of methods have been devised, including physical methods (heat, ultraviolet radiation (see Part II), and refrigeration), chemicals, antibiotics, and chemotherapeutics.

Chemical agents are very useful in controlling microbial growth and survival, and you probably have used a number of them in everyday life. One group of chemicals, classified as **disinfectants**, is used to control microbes on inanimate objects. A second group, **antiseptics**, is used for topical application to body surfaces. The third group contains disinfectants that also do some degree of cleaning, and so are referred to as **sanitizing agents**.

Most of you have probably taken antibiotics at some point in your life to control or avert a microbial infection. An **antibiotic** is a compound made by a microbe (either a bacterium or a fungus) which is inhibitory or lethal to unrelated microorganisms. There are many different kinds of antibiotics that have been discovered, but even so there is a multimillion dollar industry which is constantly screening new organisms trying to find better or new kinds of antibiotics. This continued search is crucial to public health as more microbes become resistant to current antibiotics.

Microbial sensitivity to these agents can be tested using the **agar disc diffusion** technique. In this protocol a culture of the test microbe is spread evenly over an agar plate using a swab. Paper discs containing the agents to be tested for antimicrobial activity are placed directly on the agar plate. The test compounds will diffuse out from the disc creating a gradient. When the plate is incubated, the organism will only be able to grow in parts of the plate without the compound or where the concentration of the compound is low enough that it does not affect the organism's ability to grow. The clear area of no growth around the disc is called the **zone of inhibition**; refer to Fig. 1. Microbes that are **resistant** to the inhibitory affects of a compound will be able to grow right up to the edge of the disc containing the compound.

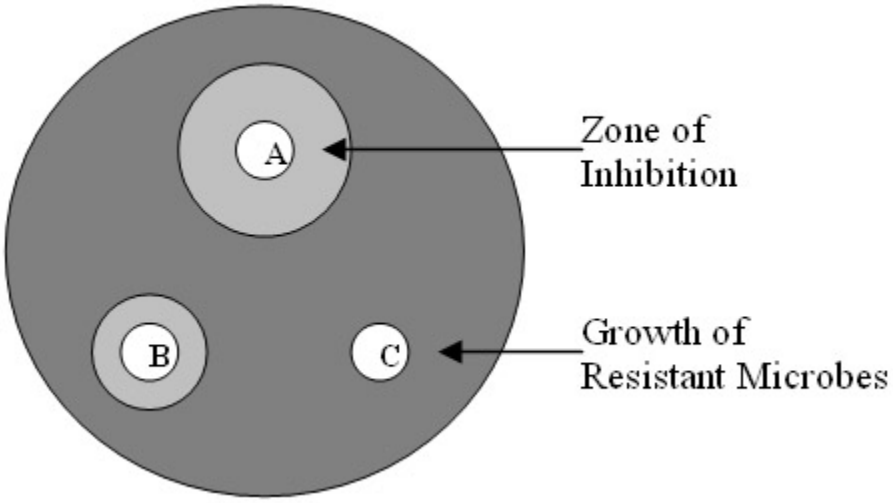


FIG. 1. Agar disc diffusion test for microbial sensitivity to antibiotics, disinfectants, antiseptics, or sanitizing agents. The chemical on disc A has a larger zone of inhibition than the chemical on disc B. The chemical on disc C has no inhibitory effect upon the growth of microbes, thus there is no zone of inhibition.

Objectives.

You will perform this lab on 4 separate days. Two portions of the lab will be performed individually, and the remainder of the lab will be performed in a cooperative learning group to achieve the following goals:

1. Inoculate an agar plate with your normal skin flora.

2. Test the antibacterial activity of some antibiotics and a number of chemical agents present in common products, such as mouthwashes, soaps, and antiseptics, against a bacterium found in the native flora of your skin. (Suggestion: select one "natural" product, one antibiotic, and any three other substances.)

3. Work as a part of a group to design and carry out an experiment that will test how a factor you have selected affects the ability of UV radiation to inhibit microbial growth.

4. Interpret the results of your individual and group experiments and discuss and present your results in oral and written form.

Materials.

Part I.

- 2 tryptic soy agar plates
- Sterile filter paper discs
- 1 marking pen
- 1 forceps
- 1 microfuge tube with sterile saline
- Alcohol jar
- Sterile toothpicks
- 1 laboratory burner
- 1 sterile swab
- 37^{o}C incubator
- Metric ruler
- Assorted antiseptics, disinfectants, antibiotics, soaps, and cleansers (this includes an item or items brought from home you'd like to test)

Part II.

- UV radiation source
- Tube rack
- Sterile swabs
- Plastic wrap
- Tryptic soy agar plates (2 or 3 per group)
- Cardboard or tinfoil
- Culture of *Serratia marcescens*

Safety Issues.

You will be working with live cultures. Normal skin flora may contain potential pathogens such as some *Staphylococci*, so care should be taken in handling these organisms. In the laboratory it is a good idea to treat all cultures as if they are pathogenic. Lab coats and safety glasses are required for this lab exercise. The instructor will demonstrate proper aseptic technique, and all contaminated materials should be disposed of in the proper designated containers. Your work area should be disinfected prior to and after you complete your lab work. Laboratory burners are an open flame, so long hair should be tied back and care should be taken to keep clothing, paper, and limbs out of the flame. Part II will involve the use of UV radiation that will be handled solely by the instructor.

Procedure.

Part I. Chemical control

- Day 1. Individually in lecture

1. Inoculate a tryptic soy agar (TSA) plate by touching it with your fingertips or any exposed skin.

2. Label the BOTTOM of the plate with your initials, the date, and your lab section number.

3. Place your plate with the lid facing down (inverted) on a tray at the front of the room that is labeled with your lab section number.

4. The plate will be incubated at 37^{o}C for 24 to 48 hours and then stored at 4^{o}C until lab.

(Why incubate at 37^{o}C? Hint: what is 37^{o}C in ^{o}F?)

5. Decide upon a household item that ***you must bring to the next lab*** to test its antimicrobial effectiveness against your natural flora.

- Results from Day 1. Individually in lab

1. Observe your skin flora plate. Does the plate of your skin flora have a lot of colonies? Do most of the colonies look the same or are there many different kinds? How would you describe the colonies you see? (Suggestions: what color is it? What shape is it? How big is it?)

2. Compare your plate to the plates from the others at your table. Do the colonies on your plate look similar to or different from the colonies on other people's plates?

3. What did you expect to find on the plate you inoculated in lecture? Did your results surprise you?

- Day 2. Individually in lab

1. You need to select one **isolated colony** from the plate you were just observing to be your test organism for this part of the experiment.

2. With flame-sterilized forceps, carefully remove a sterile toothpick from its container. Use the sterile toothpick to scrape the selected colony from the plate and transfer it to the microfuge tube with sterile saline. Mix thoroughly by capping the tube and shaking or flicking it to suspend the colony in the saline. Break up any clumps with a sterile toothpick, because you need an even suspension.

3. Using a marking pen to write on the BOTTOM of the plate, divide the plate into quadrants. This will allow you to test five different compounds by placing a disc in each quadrant and one in the center. Each disc should be well separated. Number each quadrant. Label the bottom of the plate with your initials and section number.

4. Aseptically insert a sterile swab into the tube containing your selected colony to wet the swab, and then press the swab against the side of the tube to remove excess liquid. Thoroughly cover the surface of your TSA plate with your test organism using the sterile swab to spread your suspension.

5. The following technique will be demonstrated by your instructor: using alcohol-sterilized forceps, place either an antibiotic disc or a filter paper disc soaked in the chemical agent of your choice in each of the assigned spots (see figure below). Be sure that the discs are thoroughly soaked but **well drained**, otherwise fluid will flow on the surface of the plate and obscure your results. You must flame-sterilize your forceps each time you get a new disc! Record the antibiotics and products you used (including the active ingredient) in Table 1 below.

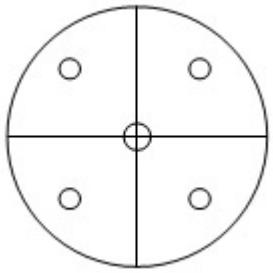


Table 1. Agar disc diffusion test

Quadrant	Product/Antibiotic	Active Ingredient	Zone of Inhibition (mm)
1			
2			
3			
4			
Center			

6. Invert the plate and place it in the tub labeled with your section number. The plate will be incubated at 37^{o}C for 24 to 48 hours. Any zones of inhibition will be measured with a metric ruler to the nearest millimeter during the next lab period.

7. Clean up your area as follows:

- Place your swab in the jar for swabs which contains disinfectant.
- Dispose of your old petri plate and microfuge tube in the plate discard container.
- Return items to where they were found.
- Disinfect your area.
- Return your lab coat and safety glasses.
- Wash your hands.

Part II. Physical control

Light from the sun includes not only visible light, but also ultraviolet (UV) radiation. There are three types of UV radiation grouped by wavelength size—UVA, UVB, and UVC (listed from the longest to the shortest wavelength). The shorter the wavelength, the more harmful the radiation but the less it is able to penetrate. UV radiation is harmful because it has a **mutagenic** effect on DNA; thus it can have major effects on living cells and, depending on the dose, is often lethal. The earth's atmosphere absorbs most of the sun's UV waves thereby preventing their full intensity from reaching the earth's surface. Without this atmospheric protection, earth would be inhospitable to much of life as we know it. In controlled situations, we can capitalize on this harmful effect by using UV radiation as a microbicidal agent. In this case, the source of the UV radiation is a germicidal lamp. Because UV radiation passes readily through air, slightly through liquids, and only poorly through solids, the object to be disinfected must be directly exposed to it for full effect. Germicidal lamps have been used in hospital rooms, operating rooms, schools, food preparation areas, nursing homes, and military housing to reduce the concentration of airborne microbes. Treatment of liquids requires special equipment to spread the liquid into a thin film that is exposed directly to a lamp. A major disadvantage of UV radiation is its poor penetration through solids, even paper. Additionally, overexposure of human tissues to UV radiation can cause sunburn, retinal damage, skin wrinkles, and cancer. The effects of UV radiation are easy to observe with microbes since they are single-celled organisms and therefore even short doses of UV radiation can cause deleterious damage to their DNA. In this lab, you will design an experiment to investigate the effect of UV radiation on *Serratia marcescens* cells and determine a way to test the effectiveness of a UV protective method or some other variable that affects UV exposure.

- Day 2. Cooperative learning group in lab

You will be working in a group of three. Establish who will be the taskmaster, gopher, and recorder for today's lab.

Brainstorming your experimental design

1. In your group, the taskmaster should lead your group to **brainstorm factors that might affect the action of UV radiation**. (Hint: remember UV exposure is harmful to our cells as well; how do we **avoid** or **control** exposure? List all ideas; be creative!) You have 5 minutes to complete the brainstorming.

2. Pick one of your ideas from #1 for development.

   a. Identify the question you are trying to answer.

   b. What is your hypothesis—that is, what do you expect to happen to your *S. marcescens* cells?

   c. What is the variable to be tested?

3. Design your experimental procedure, including the **proper controls** (Hint: in many cases you can adequately design this experiment using only two petri dishes). Check the materials list to see what will be available for you to use. **Your group is responsible for bringing at least one item to the next lab period to test for its ability to block UV radiation.** UV exposure (irradiation) will be accomplished by exposing petri plates that have been swabbed with *S. marcescens* to UV light for 5 minutes in a biosafety hood (sterile environment) without the lid on, because the lid blocks UV light. A 5-minute exposure is adequate to kill *S. marcescens* cells. Your instructor will supervise this step. Think about how you will introduce the variable you intend to test.

4. The recorder should record the following: (i) your hypothesis, (ii) a materials list, and (iii) a list of steps explaining how you will test the hypothesis.

5. The gopher should show this information (from step 4) to your instructor for approval before leaving lab.

- Day 3. Cooperative learning group in lab

Analyzing your results from Day 2 (individual) and class discussion

1. Measure the zone of inhibition to the nearest millimeter with a metric ruler. Complete Table 1 that you began to fill in on Day 2. Compare your results to others in the lab—were they the same or not? If one compound had a larger zone of inhibition than another on your plate does this mean that the first compound is more effective at inhibiting bacterial growth than the second?

2. When finished with your petri plate, dispose of it in the designated discard container.

Executing your designed experiment (group)

1. The gopher should get the materials you need to conduct your experiment.

2. Use the materials to carry out your experiment as designed. The taskmaster should make sure that all group members are contributing to performing the experiment. Your instructor will explain the safety precautions for the UV source and will be responsible for the actual irradiation process. Your plates will be irradiated for 5 minutes without the lid on.

3. Clearly label the bottom of the plates with your initials, lab section number, and treatment (+UV or no UV). Invert and place the plates in your drawers for room temperature incubation.

4. The gopher should return all unused and common materials to where they belong.

5. Clean up your area, disinfect the area, and wash your hands.

- Results from Day 3—group learning lab and class discussion

1. Design a table and record the results from your UV radiation experiment in the space below. For a given treatment, either record the actual number of colonies or devise a scale to indicate thickness of growth if there are too many colonies to count (for example, + is light growth and +++ indicates heavy growth).

2. Did your controls give the expected results? If not, speculate why the results were different.

3. Did your results support your hypothesis? If not, speculate why the results were different.

4. List two different factors tested by other groups? In general, what were their results?

Factor tested	Results

Instructor Version.
Part I of this exercise, the standard disc diffusion assay, is conducted individually while Part II, investigating the effects of UV radiation, is completed in groups of three. Part I is carried out over Days 1, 2, and 3. Part II is carried out over Days 2, 3, and 4.

Day 1

Part I. Students need to inoculate a tryptic soy agar (TSA) plate with normal flora prior to the start of this exercise. Inoculation is done during the preceding lecture session as the concept of bacterial growth is introduced. For a lecture of 50 students, this procedure takes about 10 minutes. A TSA plate for each person and several marking pens are required. This could easily be done at the end of a preceding lab period as well.

Day 2

Part I. Growth is observed and compared to other student's results. Colony description and other related concepts are covered through discussion. Aseptic transfer, swabbing for a lawn of bacterial growth, and flame sterilization of forceps are demonstrated before students pick a colony of their choice for the disc diffusion assay. It is important that students appreciate aseptic technique with their normal flora before using *Serratia marcescens* in Part II. Several different household cleaning products and antibiotics are provided and students are encouraged to bring in their own products. Trial or travel size products work well. If large containers are used it is easiest to fill the cap and dip the disc into the cap. Examples of products tested include antibiotics, kitchen and bathroom cleaners, antiseptics, mouthwashes, various spices, and other natural antimicrobials. The plates can be refrigerated after zones of inhibition are established. Students may need assistance in finding the active ingredient in the products tested. One hour of lab time is required for this portion.

Part II is introduced with a discussion about UV radiation and its effect on living cells. Groups of three students brainstorm ideas of what could protect living cells from UV radiation or enhance its effects. Ideas are brought back to the class for discussion. The goal is to design an experiment that will involve exposure of *S. marcescens* to UV radiation under various conditions. *S. marcescens* was chosen because its red color (at room temperature) shows dramatic results between growth and no growth. Additionally, the UV radiation will successfully kill the unprotected *S. marcescens* with 5 minutes of exposure.

Each group decides on a protocol for the next lab period and determines what supplies will be needed beyond what is listed in the lab manual. Protocols should be shared with the instructor so that groups can be led in slightly different directions if two groups propose the same design. This also allows time for discussion of proper controls. Additionally, supply lists can be generated and procurement responsibilities assigned. We keep a supply of sunscreen, fabrics, and other odds and ends in case students forget to bring these in. Ideas that have been tested include: sunscreens of different SPF ratings, sunscreens of different ages, different products with identical SPF ratings; clothing of different colors, textures, thicknesses, or dryness (i.e., wet versus dry); types of glass or lens materials; depths of water or distance from UV source; and length of UV exposure. At least 30 minutes is required for this portion, but the amount of introductory material covered, time allowed for discussion, and number of groups can greatly affect this.

Day 3

Part I. Disc diffusion plates are observed for zones of inhibition. Discussion of the results leads students to understand susceptibility and resistance to antimicrobial agents. While only a qualitative assay, differences between cultures and agents emerge. Students are often surprised by the "lack of activity" of some products that make claims of having antibacterial capabilities. Minimum time allotted for this activity should be 30 to 45 minutes.

Part II. To decrease the number of plates required, TSA plates can often be subdivided for application of different factors. Reminders regarding proper labeling on the bottom of the plate, not the top, will ensure proper recording of results. Clear plastic wrap is provided for those groups testing lotions. After swabbing the plate, remove the lid and cover tightly with plastic wrap. This will prevent contamination while providing a surface for lotion application. After exposure the wrap can be removed and the lid replaced. To avoid safety concerns, exposure to UV radiation occurs in a biosafety hood operated by the instructor, but students are allowed to place their plates in the hood and are then shown how the hood operates. The petri dish lids **must** be removed before exposure as the plastic will block UV radiation. If a safety hood is not available, a hand held UV source can be substituted provided a safe covering for the exposure, such as an aluminum foil tent, is available. Setting up and carrying out the experiments generally takes 30 to 45 minutes.

Day 4

Part II. Students sometimes struggle with enumeration as growth results range from one colony to lawn growth. Noticing one or two colonies on an unprotected plate leads nicely into a discussion of mutation and mutants. A brief oral report of each group's results helps to widen the scope of the discussion of the results of UV exposure.

Safety Issues.

- Normal skin flora may contain potential pathogens, such as some *Staphylococci*, so care should be taken in handling these organisms. As an alternative, a pure culture could be provided; but then students lose the opportunity to observe their own flora and they are generally more interested in testing the various antiseptics, disinfectants, etc., when it is against their own bacteria.
- Because they are opportunistic, *Serratia marcescens* may pose potential health risks and may not be suitable for, or permitted in, all laboratory settings. Alternative microorganisms may be used; however, the 5 minute kill time should be reevaluated.
- Lab coats and safety glasses must be worn during the laboratory periods.
- Lab benches should be wiped with disinfecting solution before and after use.
- Hands should be thoroughly washed after handling laboratory materials.
- Laboratory burners are used in Part I, so open flame precautions are necessary.
- We recommend that the instructor operates the UV lamp to avoid potential exposure risks.

ML Safety Statement regarding Environmental Isolates

The Curriculum Resources Committee recognizes that isolated organisms can be a powerful learning tool as well as a potential biological hazard. We strongly recommend that:

- Environmental enrichment laboratories should only be performed in classes in which students have been trained to work at a BSL2.
- Direct environmental samples (eg. soil, water) which are known to contain infectious organisms should be handled according to the biosafety level of that infectious agent.
- Cultures of enriched microorganisms, derived from environmental samples, should be handled using Biosafety Level 2 precautions.
- Mixed, enriched or pure cultures of microorganisms from environmental samples with a significant probability of containing infectious agents should be manipulated in a biosafety cabinet if available.
- Where possible, media used for the enrichment of environmental isolates should contain an appropriate anti-fungal agent.
- Instructors should be aware if they are teaching in regions with endemic fungi capable of causing systemic infections, and should avoid environmental isolations.

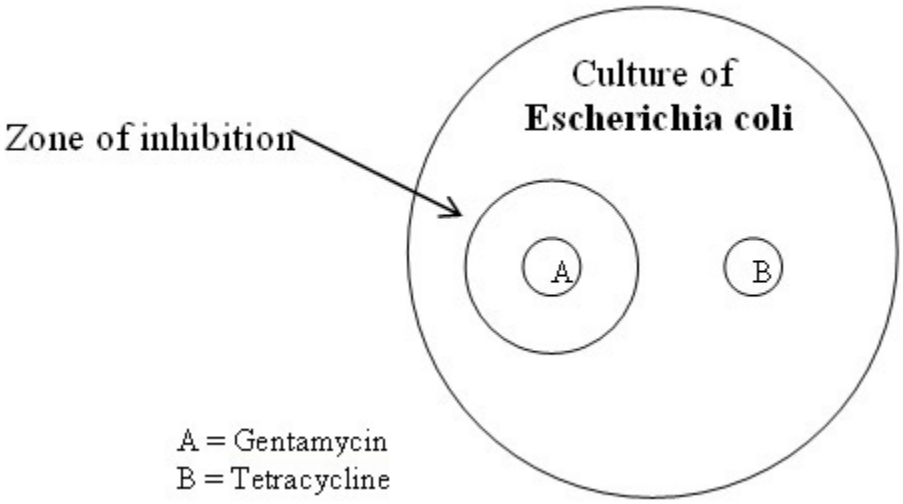
ASSESSMENT AND OUTCOMES

Suggestions for Assessment.
Group and individual assessment is accomplished through class discussions, a written group report for Part II (see appendices), and quizzes. Examples of quiz questions we have used:

1. _____ are chemical agents used to control microbes on inanimate objects.

2. Give one example of a physical method for controlling growth of microorganisms.

3. Given the information below, is tetracycline effective at inhibiting the growth of *Escherichia coli*? Explain why or why not.



4. Given the above information, can we tell how effective gentamycin would be at inhibiting *Staphylococcus aureus*. Explain your answer.

5. Sometimes a chemical product which caused a large zone of inhibition on one person's plate, caused only a small or no zone of inhibition on another person's plate. What is one possible explanation for this?

6._____ is a compound made by a microbe which is inhibitory of or lethal to unrelated microorganisms.

7._____ is the molecule that ultraviolet radiation acts upon, causing mutations and often death of the cell.

8. You have a plate which you swabbed completely with *Serratia marscescens*, you then remove the lid and cover half the plate (A) with cardboard leaving the other half (B) uncovered. After exposing the plate of *Serratia marscescens* to UV radiation in the sterility hood for 5 minutes, you incubate the plate at room temperature for 24 hours. Which half of the plate will have growth and why?

9. Why do you have to remove the petri plate lid when exposing cultures to UV radiation?

Field Testing.
We have used this lab in our general education nonmajors microbiology class for the past 10 semesters. Each lab section has 15 to 24 students from diverse academic backgrounds such as business, music, computer science, nursing, and elementary and secondary education. Four different faculty members have taught this lab and have found that it is easily tied to student concerns in everyday life such as, "Do the new waterless hand gels really work?" and "Is there really no difference in protection between SPF 30 and SPF 45?" In classroom surveys, this lab has been identified by some students as their favorite with reasons including:

"I liked seeing what was on my hand."

"We got to design it ourselves which made it more interesting."

"We were allowed to be creative and design our own lab procedure."

SUPPLEMENTARY MATERIALS

Possible Modifications.
1. Rather than using bacteria from the skin flora, repeat the chemical control lab using bacteria from a mouth or nasal swab. Do the mouthwashes and natural products, such as garlic and cinnamon, appear to work better against these bacteria? Do the hand sanitizers perform better against skin, oral, or nasal flora?

2. Subculture one of the resistant bacteria from your chemical control plate (either one growing right up against a chemical disc or a mutant found growing within a zone of inhibition). Test this organism against a variety of products to see if it has multiple resistances (particularly interesting with different antibiotics).

3. Rather than using plates, the experiment could be done with a tube dilution assay allowing for quantitative results. If the tubes are read with a spectrophotometer, these results could be graphed to show a death curve.

4. Repeat the UV experiment using different types of bacteria to explore the variety of UV resistance that exists. *Deinococcus radiodurans* is a nice inclusion if available.

References used and other useful references

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4. **Nester, E. W., D. G. Anderson, C. E. Roberts, Jr., N. N. Pearsall, and M. T. Nester.** 2004. Microbiology: a human perspective. McGraw-Hill, Boston, Mass.

Appendices and Answer Keys.

Physical Control of Microorganisms: Group Results

Names of Group Members _____ Date: _____

Instructions. Staple the following items in order to this sheet: (i) hypothesis and (ii) how you executed your experiment. This must be written in enough detail that someone could follow your directions to repeat your experiment! Only one completed set of materials should be turned in per lab group.

1. Design a table and record the results from your UV radiation experiment in the space below. For a given treatment, either record the actual number of colonies or devise a scale to indicate thickness of growth if there are too many colonies to count (e.g., + is light growth and +++ indicates heavy growth).

2. Did your controls give the expected results? If not, speculate why the results were different.

3. Did your results support your hypothesis? If not, speculate why the results were different.

4. List two different factors tested by other groups? In general, what were their results?

Factor tested	Results

Answers to quiz questions

1. Disinfectants and/or sanitizing agents
2. Examples include: temperature (heat or cold), radiation (UV, gamma), filtering
3. No, there is no zone of inhibition around the disc containing tetracycline.
4. No, because different organisms can have different susceptibilities to antibiotics. The fact that gentamycin inhibits *E. coli* does not tell us anything about its affect on *S. aureus*.
5. As each student used an isolate from their normal flora, most likely they are testing different organisms and different organisms can have different susceptibilities resulting in different-sized zones.
6. Antibiotic
7. DNA
8. Side A, because the cardboard cover blocked the UV radiation which would otherwise have killed the cells as it did on side B which will have no growth.
9. The plastic of the petri plate lid will block the UV radiation. So, if the lid is left on, the culture will not actually be exposed to UV radiation.