Revised 8/05

Biology 100

Effectiveness of Sunscreens Against Ultra-Violet Light

Introduction:
With enough exposure, ultra violet (UV) light is lethal to bacteria. Ultra violet light may also cause damage to human skin cells. It is thought this damage may destroy the ability of the skin cells to control cell division, thus leading to a type of skin cancer called melanoma. For this reason people are encouraged to protect their skin from the potentially harmful effects of UV light by using sunscreen creams. Sunscreen creams have different Sun Protective Factors (SPF). You may purchase sun block with SPF values of 15, 30, or 45. The higher SPF values, in theory, provide longer protection from UV light. In today's laboratory we will design experiments to compare two different kinds of sunscreen (SPF 15 vs. SPF 30; or current sunscreen vs. expired, etc.) to evaluate their effectiveness at protecting bacteria from the effects of UV light.

Because it is unethical to use humans to conduct scientific experiments, we will use *Escherichia coli (E. coli)*, strain K-12, a type of bacteria, as a substitute for human skin in today's experiment. This is called a model. We will test the efficacy of sunscreen to block UV light on bacteria, and then we may use that information to consider the efficacy of the same sunscreen on human skin cells. In this experiment each group will formulate its own question and hypothesis regarding the effectiveness of a variety of sunscreens to protect bacteria from cell death.

Ask a Critical Question:
State the question that you are asking in this experiment (D*o sunscreens with higher SPF values provide greater protection?; Do different brands of sunscreen provide greater protection?; Is duration of exposure for different brands a factor?, etc*). This statement will become the purpose of your experiment and written in the Introduction section of your lab report.

Hypothesis: State your hypothesis.

Materials and Methods:
This is a two-part experiment. During the lab period you will formulate your question and hypothesis, inoculate Petri dishes with bacteria, and then cover the dishes with Saran wrap that has a covering of sunscreen. You will then expose your samples to UV light. In the next class period, you will perform a colony count of those *E. coli* that survived exposure to the UV light. This is necessary to calculate the percent survival of the bacteria. The way you design and conduct your experiment may be slightly different from

your classmates, however, the basic procedure will be the same for all. Be clear about the question you are asking, and the design of the experiment before you start.

Escherichia coli strain K-12 is not a harmful bacterium, however, you should wash your hands before and after handling *E. coli*, and you should use care not to spill any of the sample while conducting your experiments. Contact your instructor should you spill anything.

Three important concepts that you must understand for this experiment:

Control: A scientific control is that component of the experiment that permits comparison of results. You need to know how many cells would have grown if they had not been exposed to the UV light. Covering half of the Petri dish with aluminum foil will block the UV light and not allow it to reach the growing cells. This will permit maximum cell growth and represents a control. It is also important to control for the experimental procedure. UV light is expected to cause bacterial cell death, but this must be verified in order to accurately state that any application of sunscreen prevents UV-induced cell death. Therefore, Petri dishes where no sunscreen is applied should be used as a second control. (Half of each of these plates will still be covered with aluminum foil.) Another question to consider is whether the mere application of sunscreen, regardless of SPF value, does, in fact, prevent UV-induced cell death. To control for this, Petri dishes where lotion is applied to one half of the dish should be used.

Experimental: Covering the other half of the Petri dish with sunscreen may only reduce the passage of UV light reaching the cells, causing some cell death and reduced growth. This is your experimental group. In this experiment you will have two experimental groups where you compare one type of sunscreen with another. Because in all cases half of each dish will be covered with foil, we are allowing each Petri dish to serve as its own control.

Sample Size (N): One Petri dish represents a sample size of only one (N=1). This gives you no reliable basis to judge your experiment. You should use at least three Petri dishes (N=3) for each test group to obtain reliable results. Therefore, your experiment will require a total of 12 Petri dishes: 3 for a no coating control, 3 for a lotion control, 3 for one sunscreen and 3 for the other. In each case, each experimental dish will serve as its own control.

Lab Period 1

Experimental setup:

1. After your class or lab discussion of the scientific method, your group will brainstorm an experimental design to test the relative effectiveness of two sunscreen products available to you in the lab. Your group must fill out the Scientific Method design sheet that follows the lab explanation for your instructor's approval before you get your tube

of bacterial culture. Be sure to record your final lab design so you will have to write the procedure section of your paper.

2. Each group will have a number of fresh Petri dishes at their bench, which used to evaluate the growth effects of one sunscreen versus other sunscreen. These plates contain the growth media tryptic soy agar (TSA). This agar contains all the nutrients necessary for bacterial growth.

3. Do not remove the lid of the Petri dishes. Label the **bottom** of your Petri dishes with your group name and the date, and then divide the plate in half with a line. It is best to label along the edge of your Petri dish so as to not obstruct your view of the bacterial colonies that will grow once the procedure is complete. Label one side of the line 'C' for control (foil side), the other side with 'E' for experiment (non-foil side). Label three of the dishes as "uncovered", three as "lotion," three with the first type of sunscreen you are testing and then the remaining three dishes with the other sunscreen you are testing. These will represent your two experimental treatment groups. Remember, half of each plate will be covered with foil and serve as its own control.

4. Put on gloves. Your bacteria will be supplied to you in a liquid, broth culture. The bacteria have already been diluted so that you should get about 100 colonies on your control plate. The tube will be clear but there are many bacteria in there. It is a good idea to first gently flick the bottom of the test tube to evenly distribute the bacteria throughout the culture medium. While holding the lid to avoid contamination, pipette 0.1 ml of bacteria from the sample into each Petri dish. Spread the sample evenly with a sterile "hockey stick" throughout the entire dish. This is called a bacterial lawn. Quickly put the lid back in place, and dispose of the hockey stick in the jar of Vesphene provided.

5. Cut pieces of Saran wrap large enough to cover the top of each Petri dish. Remove the lid and cover the top of the Petri dish with the Saran wrap. Snuggly fold the edges beneath the bottom of the Petri dish. Secure the edges of the Saran wrap with masking tape. Keep the Petri dish lids for later use.

6. Using a balance, weigh out (*unless of course you are testing the efficacy of different amounts of the same sunscreen*) 0.5 g of sunscreen for each Petri dish. Be sure to tare the balance prior to each weighing, and tare the balance with the weigh boat on it. Apply the sunscreen evenly to the Saran wrap using gloved fingers. Try to use the same application technique for each dish.

7. Cut pieces of aluminum foil large enough to cover the control half of each Petri dish. Fold the foil over one half of the plate using the line drawn previously.

Methods:

1. Locate the UV lamp you will be using for the experiment. **Note the wavelength in nanometers, and note the time of exposure necessary to kill the bacteria**. This information will need to be reported in your lab report.

2. Expose your Petri dishes to the UV light using the same technique (duration, position, and distance from light source) for each.

3. Once your samples have been exposed to the UV light, remove the aluminum foil and the Saran wrap. Put the Petri dish lids back on.

4. Stack your plates into groups of three and tape them together with the masking tape. Make sure your group name is clearly written on the masking tape. Place them, **inverted**, into the carriers provided. The plates are inverted because condensation will form and drip onto the bottom surface while the plates are incubated.

Period 2 (in class)

Results:

After ~16 hours of bacterial growth at 37° C, the Petri dishes will be removed from the incubator by the TMCC staff and stored in the refrigerator at 4°C. This will arrest the growth until you can complete your experiment. You will be able to answer your experimental question as to which sunscreen offers better protection by counting the number of bacterial colonies (Colony Count) present on the control and experimental sides of each Petri dish. The number of colonies represents the bacteria that survived the UV light and reflects the amount of protection the sunscreen offered.

Accurately count the number of colonies on the control side of one the three Petri dishes for your first sunscreen (treatment group). These are your raw data for the control side. Now perform colony counts on the experimental side of each of the same plate and record the raw data.

Calculate the % survival for the treatment group:

$$\text{Percent survival} = \frac{\text{Colony count with sunscreen (experimental)}}{\text{Colony count with aluminum foil (control)}}$$

Repeat the control and experimental colony counts for the remaining two plates for your first sunscreen (treatment group), and calculate the percent survival for each of these plates. Then, calculate the **average percent survival** for your first sunscreen (treatment group) by adding the three percent survival values together and dividing by 3.

Repeat the control colony counts, percent survival calculations, and average percent survival calculations for the remaining treatment groups: the second sunscreen tested, the "uncovered" plates and the "lotion" plates.

1. Prepare a table showing your raw data and averaged data for both the experimental groups and their respective controls.

2. Make a bar graph comparing the percent survival of your "uncovered" plates, "lotion" plates, and two experimental groups.

3. Write a few sentences summarizing your results from the table and graph. Do not interpret your results here.

Discussion:
This is where you will explain your results from the data and the graphs you have made. Was your question answered? Was your hypothesis supported or not? Compare results in control and experimental groups. Do you have confidence in your results? State your conclusions. Can we use bacteria as a model to explain the effects of UV light on human skin cells? Why or why not? Suggest further experiments that may help clarify your results.

Assignment:
You will turn in a formal scientific write-up of your experiment according to the guidelines discussed in lab. Your write-up should include a table of your raw data, a table of calculations for percent survival and average percent survival, and a bar graph comparing the average percent values of each of the treatment groups: "uncovered", "lotion", and two sunscreen groups. **Each student must complete and submit this write-up individually**.

Scientific Method Worksheet

Use the following pages to help you plan out your experiment. It is also a good idea to keep an individual copy to help you write your formal report.

Question:

Hypothesis:
What do think the answer to your question will be? What do you predict will happen in terms of protecting the bacterial cells from death by UV exposure?

Controls:
What are the variables that you will hold constant throughout your experiment? Also, since you will be testing sunscreen effectiveness in some capacity, what control experiments do you need to perform to ensure that UV really does kill bacteria and that it is the SPF in sunscreen that is the protectant?

Tests:
Which variables will you be testing, i.e. how are you going to answer the question that you proposed?

Procedure (Experimental Set-up and Methods):

What is the step-by-step process that you will be following in order to carry out your experiment? How much sunscreen will you use? How will you apply the sunscreen? How many TSA plates will you need? How much bacteria will you use, and how will you apply it to the TSA plates? What systematic approach will you use to expose the TSA plates to the UV light source since you will not be able to expose all your plates at once?