Death Rays: What Duration of Ultraviolet Exposure Kills Bacteria?

https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p017/microbiology/ultraviolet-exposure-kills-bacteria (http://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p017/microbiology/ultraviolet-exposure-kills-bacteria)

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Experimental Procedure

Working with Biological Agents

For health and safety reasons, science fairs regulate what kinds of biological materials can be used in science fair projects. You should check with your science fair's Scientific Review Committee before starting this experiment to make sure your science fair project complies with all local rules. Many science fairs follow Intel® International Science and Engineering Fair (ISEF) regulations. For more information, visit these Science Buddies pages: Projects Involving Potentially Hazardous Biological Agents (http://www.sciencebuddies.org/science-fair-projects/project_src_biological_agents.shtml) and Scientific Review Committee (http://www.sciencebuddies.org/science-fair-projects/project_src.shtml). You can also visit the webpage ISEF Rules & Guidelines (http://www.societyforscience.org/Page.aspx?pid=312) directly.

This science fair project involves the use of the bacteria *E. coli*. While *E. coli* is not considered a biohazardous or dangerous bacteria, it is important to always properly clean and dispose of bacteria and supplies that come in contact with it. See the *Bacterial Safety* guidelines for more details on how to handle bacterial cleanup and waste.

Safety Note: Adult supervision is required for this project. Read and follow these Ultraviolet Light Safety Precautions (IBC UMN, 2003):

- The germicidal ultraviolet (UV) light used in this project will also damage unprotected human cells. Your eyes and skin are particularly susceptible to UV damage.
- Exposure to UV light can burn the retina or irritate the cornea and the conjunctiva. This can cause a feeling of "sand in the eye" and heightened sensitivity to light. Symptoms appear from 6 to 24 hours after exposure and usually disappear within 48 hours.
- Persons who have had the lens of an eye removed (e.g. cataract surgery) can receive permanent retinal damage from UV exposure - including blindness.
- Individuals who are exposed to photosensitizing agents (e.g. some oral drugs or topically applied creams) may not be aware of heightened sensitivity to UV radiation.
- UV radiation burns skin promoting skin aging and cancer.
- If possible, the UV source for irradiating bacterial cultures should be operated remotely, so that the Investigator is not exposed to UV light.
- If this is not possible, then the UV source should be set up so as to avoid direct exposure to the Investigator (i.e., placed behind a UV-blocking barrier).
- The Investigator should also use the following personal protective equipment:
    - All skin should be protected including face, neck, hands, and arms.
    - Wear gloves and long sleeves covering all skin above the gloves.
    - Eyes and face should be protected by a face shield designed to block the UV wavelengths used. Radiation can readily reach the eyes through the open sides of standard eye glasses, so they do not provide sufficient protection.

1. Follow the directions in the kit to reconstitute the dried *E. coli*. Let the reconstituted *E. coli* sit at room temperature for five minutes.
2. Prepare 15 nutrient agar plates with the *E. coli* bacteria: while wearing gloves, gently shake the reconstituted vial of *E. coli*, add two drops of the bacterial suspension to a plate, use a sterile cotton swab to spread the bacteria around the entire plate. Cover the plate and wait 5 minutes for it to dry. Repeat for all 15 plates, using a fresh cotton swab every third plate.
3. Protect half of each plate from UV light by using aluminum foil to cover half of the lid for each plate.
4. You will use the plates in five groups of three plates each. All plates should be at the same distance from the UV source. The following table shows the suggested UV exposure time for each group of plates. Remember to read and follow the UV light safety precautions (above) while performing this step.

Group	UV light exposure time (seconds)
1	15
2	30
3	60
4	120
5	300

4. Immediately after the UV light exposure, use a permanent marker to indicate which half of each plate received UV light, and the duration of the exposure.
5. Remove the foil coverings, then incubate the plates, inverted (lid down and agar-side up), overnight at 37°C (or longer if at lower temperature).
6. Count colonies in both halves of each plate.
7. For each group of plates, calculate the average and standard deviation of the number of colonies in each half of the plate.
8. Make a graph showing the average number of colonies (y-axis) as a function of UV exposure time (x-axis).
9. On the same graph, you can also use a different symbol to plot the average number of colonies on the unexposed (control) side of each plate.
10. Is the average number of control colonies consistent across the five groups of plates? Why or why not?
11. What duration of UV exposure results in 50% bacterial mortality? Are there any plates with 100% bacterial mortality? If so, what duration of UV exposure results in 100% bacterial mortality?

Bacterial Safety

Bacteria are all around us in our daily lives and the vast majority of them are not harmful. However, for maximum safety, all bacterial cultures should always be treated as potential hazards. This means that proper handling, cleanup, and disposal are necessary. Below are a few important safety reminders.

- Keep your nose and mouth away from tubes, pipettes, or other tools that come in contact with bacterial cultures, in order to avoid ingesting or inhaling any bacteria.
- Make sure to wash your hands thoroughly after handling bacteria.
- **Proper Disposal of Bacterial Cultures**
    - Bacterial cultures, plates, and disposables that are used to manipulate the bacteria should be soaked in a 10% bleach solution (1 part bleach to 9 parts water) for 1–2 hours.
    - Use caution when handling the bleach, as it can ruin your clothes if spilled, and any disinfectant can be harmful if splashed in your eyes.
    - After bleach treatment is completed, these items can be placed in your normal household garbage.
- **Cleaning Your Work Area**

- At the end of your experiment, use a disinfectant, such as 70% ethanol, a 10% bleach solution, or a commercial antibacterial kitchen/bath cleaning solution, to thoroughly clean any surfaces you have used.
- Be aware of the possible hazards of disinfectants and use them carefully.