

ULTRAVIOLET KILLING OF BACTERIA

page 49a

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Ultraviolet light induces the formation of covalently-linked pyrimidine dimers in exposed DNA. If uncorrected, these dimers can trigger changes in base sequence during subsequent replication and thus induce genetic mutations.

In human beings, these mutations are responsible for the high incidence of skin cancers among persons who spend extended periods of time unprotected in the sunlight or tanning beds. In bacteria, they are responsible for the germicidal effect of UV light. This latter effect will be demonstrated in this experiment, but the importance of protecting one's self from the genetic damage of UV light should be apparent as well. Yes, tanning beds are capable of causing this genetic damage.

EQUIPMENT:

UV transilluminator (or UV "Sunlamp") ¹	sterile 0.1 mL pipettes
If "Sunlamp": 2 ring stands and cross bar with clamps	stopwatch
Nutrient agar plate (etc.)	opaque cardboard
2 mL melted top agar, 45 C, inoculated with <i>E. coli</i>	protective eye wear
wax pencil	37°C incubator
Bacterial cultures ² [Handle pathogens with care]	

1. MARK THE PLATES:

PLATE A: On a nutrient agar plate, draw a grid with 16 squares as large as possible on the plate bottom (four lanes in either direction). Label the three central horizontal lines as demonstrated to indicate the exposure time on either side of the lines: 135 sec/90 sec; 90 sec/15 sec; and 15 sec/0 sec. The bottom lane (0) is the control (no exposure to UV).

2. Pour inoculated top agar on it for the lawn.

3. SET UP THE UV LAMP: We will use a UV transilluminator. The plates will be placed on its surface. (See below for alternative UV lamps). CAUTION: Use UV shielding eye wear.

4. **Wearing protective eye wear**, turn on UV lamp, let warm up for one minute. (Do not look at the UV lamp, it is damaging to the rods and cones in your retinas).

5. **Remove the cover of the plate** and cover completely with a piece of cardboard. Place the covered plate directly on the UV transilluminator. (Alternatively, place plate at edge of transilluminator.)

6. **EXPOSE THE PLATE IN STAGES** to UV for 135, 45, 15 and 0 seconds as follows. (*Do not expose your hand to the lamp any longer than necessary.*) **Pay close attention!**

a. Start a stopwatch and simultaneously push the plate over the edge of the rectangular UV light source to the 3/1 line. Exposed for an initial **2.0 min.** (Expose only the 3 min. region)

b. After 2.0 minutes, move the plate to the, 1/0.3 line, expose for **40 more seconds.**

c. After 40 seconds (stopwatch says 2:40), move to the third 0.3/0 line, and expose for a **final 20 seconds** more (stopwatch will say 3 minutes when finished).

7. **INCUBATE** at 37°C for 24-48 hours, and score UV sensitivity of the various cultures tested, and illustrate the results of the exposure on bacterial growth in your notebook.

¹ For UV sun tan lamps, (such as a GE 275 watt), mount the lamp 25 cm above the surface of the desk and clamp securely with a ring stand and clamp. Expose for 9, 3, 1 and 0 minutes.

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TESTING UV SENSITIVITY OF VARIOUS STRAINS OF BACTERIA:

1. Draw a grid on the plate bottom with four horizontal lanes and five vertical lanes. Label the vertical lanes with the initials each of five cultures you choose to streak (see below). Add top agar as above, step 1

2. **CROSS-STREAKING TECHNIQUE:** Using sterile technique, dip a sterile 0.1 mL into the first O.N. bacterial culture. Around 0.02 mL should be drawn up by capillary action. Draw the pipet along the lane, viewing the agar surface by reflected light to see that the liquid is being deposited and the agar is not scraped up. About 0.01 mL will be delivered, or around 10⁷ cells, per streak. Do not cross-contaminate. Repeat for the four other strains. Replace lid as soon as possible.

² Suggested strains to test:	Bs: <i>Bacillus sphaericus</i>	Pv: <i>Proteus vulgaris</i>
	EcB: <i>E. coli B</i>	St: <i>Salmonella typhimurium</i>
	EcK: <i>E. coli K12 (λ)</i>	Sa: <i>Staphylococcus aureus</i>
	Pa: <i>Pseudomonas aeruginosa</i>	Sm: <i>Serratia marcescens</i>