Experiment 2: Effect of Chemical Germicides on Bacterial Growth

Germicides are a type of chemical means to reduce the number of microorganisms, not totally eliminate them, on a surface, in a liquid, or on a person. Disinfectants are germicides designed for use on inanimate objects (floors, countertops, instruments, etc.) while antiseptics are designated for use on living tissue. In this experiment, you will soak filter paper disks with various chemicals then place the disks on a confluent lawn of bacteria and look for a zone of inhibition (clear area) surrounding the disk. The size of the zone of inhibition is proportional to the growth inhibiting properties of the chemical.

Materials

Nutrient agar plates from Experiment 1

Nutrient agar

(4) 5 cm. Petri dishes

4 Filter paper disks

10 mL Hibiclens™ antimicrobial, antiseptic hand cleanser

Forceps

Candle

Matches

Deionized water

5 mL Sterile phosphate buffered saline (PBS)

4 Sterile, disposable transfer pipettes

4 Sterile snap-cap tubes

4 Sterile, disposable inoculating loops

(3) 15 mL Screw-top tubes

Ruler

Parafilm™

10 mL Graduated Cylinder

100 mL Beaker

*Bottle of 70% Isopropyl Alcohol

*10% Bleach

*Scissors

*You must provide

Procedure:

1. Prepare 4 nutrient agar plates as described in Experiment 1, Steps 1 - 5.
2. Use a permanent marker to label the bottoms of the plates as “Skin”, “Nose”, “Throat”, and “Shoe”.
3. Use a permanent marker to label the 4 snap-cap tubes as “Skin”, “Nose”, “Throat”, and “Shoe”.
4. Put 1 mL of PBS into each of the 4 snap-cap tubes.

5. Use a sterile inoculation loop to transfer an individual colony from the “Skin” plate from (Experiment 1) to the “Skin” tube and gently mix the loop in the PBS.
6. Use a sterile transfer pipette to evenly distribute all of the PBS from the “Skin” tube over the surface of the newly prepared “Skin” plate.
7. Use a sterile spreader to evenly distribute the PBS/bacterial solution over the surface of the agar.
8. Repeat Steps 5 - 7 for the remaining 3 plates. Remember to use a new sterile loop, transfer pipette, sample, and spreader each time.
9. Dispose of the snap-cap tubes.
10. Seal the plates with Parafilm™, invert them, and incubate them in a warm location (not to exceed 37.7 ° C or 100 °F) for 2 - 3 days, or until a confluent lawn of bacterial growth occurs. The growth will look like a cloudy film on top of the agar.
11. After the lawn of bacteria has formed, use a permanent marker to divide the plates into 4 quadrants on the back of the dish and label the quadrants “Bleach”, “70% Isopropyl Alcohol”, “Hibiclens”, and “Control”.
12. Use a graduated cylinder to measure and pour 10 mL of the 10% bleach solution in a 15 mL screw top tube. Label this tube as “10% Bleach”.
13. Measure and pour 10 mL of the 70% isopropyl alcohol in a 15 mL screw top tube. Label this tube as “70% Isopropyl Alcohol”.
14. Measure and pour 10 mL of the Hibiclens™ in a 15 mL screw top tube. Label this tube as “Hibiclens”.
15. Use scissors to cut each of the four filter paper disks into 4 even quarters (you should end with 16 total pieces of filter paper).
16. Sterilize the forceps with your candle. To do this…
    a. Pour isopropyl alcohol into a 100 mL beaker until you have a depth of 2 - 4 cm. Place the cap back on the bottle and position it clearly out of the way.
    b. Light your tea-candle and set it aside. Be very cautious that your tea-candle is safely positioned away from the beaker of isopropyl alcohol.
    c. Dip the forceps into the isopropyl alcohol for 10 seconds.
    d. Without touching the forceps to anything, carefully pass the end of the forceps through the flame several times.
    e. Extinguish the flame when complete.
17. Pick up a filter paper quarter with the forceps and dip the disk into the 10% bleach solution. Remove the disk from the solution and allow all excess bleach to drip off. Place this on the “bleach” quadrant of one of the petri dishes. Repeat this process for the remaining 3 plates.

18. Repeat Step 16 - 17 using the two other chemical germicides (Hibiclens™ and 70% isopropyl alcohol).
19. For the Control quadrant, sterilize the forceps as in Step 16. Then, place a clean, dry filter paper quarter in each of the “Control” quadrants.
20. Seal the plates with Parafilm™ and incubate them in a warm area (not to exceed 37.7 °C, or 100 °F) for 2 - 3 days.
21. Observe the clear zone of inhibition around each disk and measure with the ruler. Record your results in Table 3.
22. When you are finished with the experiment, pour a 10% bleach solution over the entire surface of each plate and incubate them for 20 minutes at room temperature. Dispose of the bleach down a drain with running water, seal the plates with Parafilm™, and dispose of them in the trash.

Table 3: Experiment 2 Results

Sample	Germicide	Zone of Inhibition (mm)	Relative Effectiveness
Skin	10% Bleach		
	70% Isopropyl Alcohol		
	Hibiclens™		
	Control		
Nose	10% Bleach		
	70% Isopropyl Alcohol		
	Hibiclens™		
	Control		
Throat	10% Bleach		
	70% Isopropyl Alcohol		
	Hibiclens™		
	Control		
Shoe	10% Bleach		
	70% Isopropyl Alcohol		
	Hibiclens™		
	Control		