

Exercise 11

(Ed. F 2010)

The Effects of Disinfectants on Bacteria

The Disk Diffusion Method

INTRODUCTION:

Student Learning Objectives: After completing this exercise students will:

1. Demonstrate the effects of disinfectants on the growth of selected bacteria.
2. Describe the concept of the disc diffusion method.
3. Demonstrate the effects of disinfectants on spore forming and non-spore forming bacteria.
4. Identify the mechanisms by which disinfectants kill or inhibit microorganisms.

Activities for today:

- Inoculate assigned bacteria onto NA plates, place paper disks saturated with disinfectants on the agar, and observe for growth and inhibition.
- Evaluate the results of the effects of the disinfectants on different bacteria.

Materials

Work in groups of 4 per table. This is a group activity. Each group needs the following:

Broth cultures of the following bacteria:

Bacillus subtilis, *Pseudomonas aeruginosa*, *E. coli*, and *Staphylococcus aureus*

4 Nutrient Agar plates

3 ml sterile saline tubes to be used as control 1 tube per table.

Sterile cotton swabs

Sterile filter paper disks

Forceps

30 ml alcohol in 50 ml beakers (to be used to flame the forceps)

10% Bleach/water solution in a test tube

Citric Acid based disinfectant/Citric Acid Shower Cleaner

Surface disinfectant used in lab

Quat (Ammonium based disinfectant) 10% solution

Ruler (metric)

Determining the effects disinfectants on bacterial survival

Many classes of disinfectants are used to kill or inhibit microorganisms. The choice of disinfectant is based upon many factors, such as type of material that needs to be disinfected, organic versus inorganic surfaces, and the effective concentration of the disinfectant. In this exercise, you will test the effects of several classes of disinfectants using the disk diffusion method, on the survival of different organisms encountered in hospitals, or in household settings (Kitchen, bathroom, etc). Due to the time factor and resource allocation, you will work with your labmates to investigate the effectiveness of these disinfectants against bacteria only. Teamwork is important in this exercise, as it will save you time and effort. Questions to keep in mind: What is the most effective way to use these disinfectants? Are these disinfectants effective against all types of bacteria equally? How are these disinfectants used in everyday activities?

In this exercise, you will using the disk diffusion method to determine which disinfectant is most effective or least effective against bacteria. Use the aseptic technique to avoid any contamination of the media and equipment. Remember, treat all bacteria in lab as pathogens at all times.

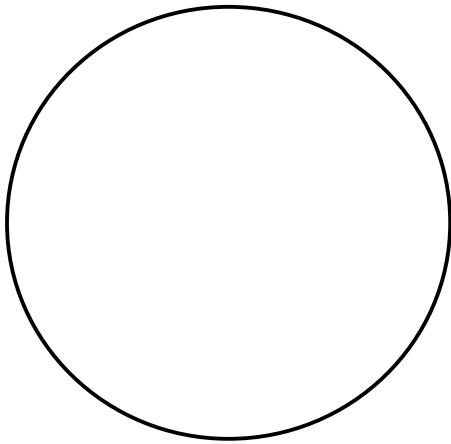
Procedure:

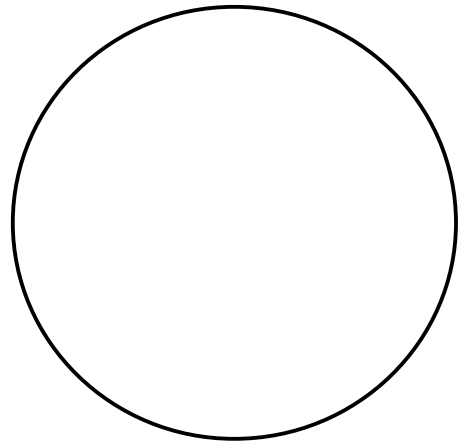
1. Using a marker, label 4 NA plates with the names of the bacteria being evaluated. Draw 5 lines on the bottom of each plate dividing it into 5 sectors.
2. Label each sector with the name or number of each of the 4 antiseptics. Label one sector "Control".
3. Using a sterile swab, dipped in the selected bacterial culture, inoculate the corresponding NA plate to obtain confluent growth (lawn growth).
4. Dip the forceps about 1 inch deep in the beaker containing the alcohol.
5. Holding the forceps at an angle, pass it through the flame of the bunsen burner. Keep the alcohol beaker away from the Bunsen burner flame.
6. Cool off the forceps.
7. Using the sterile forceps, pick up a paper disk from the container, dip it in the disinfectant and place it on the agar in the corresponding sector.
8. Do this for all 4 disinfectants, and lastly use the saline solution for the "Control".
9. Repeat steps 3-8 for all four bacterial species.
10. Incubate both plates for 24 – 48 hours and check for growth and zones of inhibition.
11. Complete the table and draw the plates below.

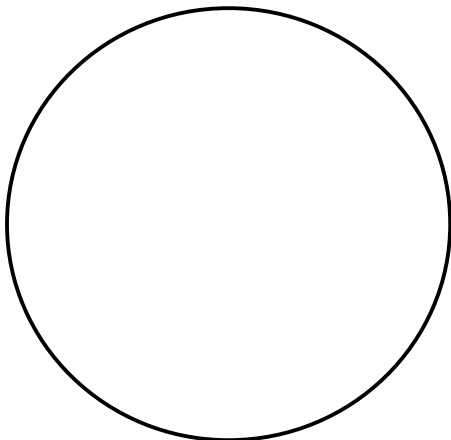
Results – Zone of inhibition measured in mm

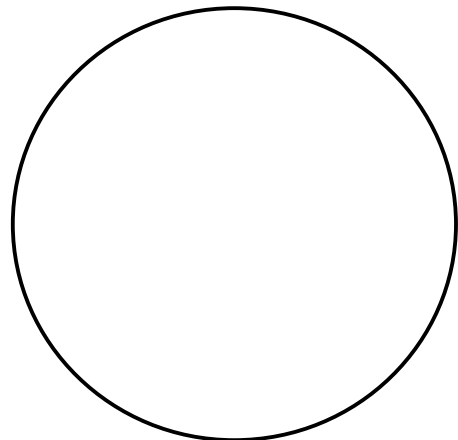
Disinfectant	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>
Control				

Draw the results of the TSA plates below, and indicate the applicable information.









General Questions

1. Based on the results in the table above, which was the most effective disinfectant? Why?

2. Based on the results in the table above, which was the least effective disinfectant? Why?

3. Would using the most effective disinfectant on all types of material be practical? Why?

4. Based on the aggregate results of the exercise (all bacterial species) and chapter readings, list some of the factors that can affect the efficacy of these disinfectants:

5. Why would you use a Control disc? Was this a positive or negative Control?
