Efficacy of antiseptics and disinfectants on the control of microbial growth.

Since the advent of the germ theory of disease, the need for controlling microbial populations in certain settings has been increasingly evident. Chemicals include agents which destroy membranes, denature proteins or disrupt cell walls. To test the effectiveness of a chemical as a bactericidal or bacteriostatic agent, many methods are used. A simple method to compare the effectiveness of chemical agents is the paper disc method. A sterile disc of filter paper is impregnated with a chemical agent and placed on a plate of nutrient medium that has been seeded with a test organism. The plate is incubated for 24 hours and is observed for clear zones of inhibition (zones of no growth) surrounding the disc. Under standard conditions, where variables such as concentration and diffusion rates of the agents tested are known, the size of a zone of inhibition can be used to infer the agent’s effectiveness against a specific organism and can be compared quantitatively against other substances.

Antiseptics are chemical agents that are usually bacteriostatic because they are intended for contact with living tissue. An antiseptic must be effective against microorganisms without harming the sensitive tissue which has been invaded by them, thus they cannot be as harsh as disinfectants. Disinfectants are chemicals that are generally bactericidal and are used primarily on inanimate objects.

In this exercise you will expose various organisms to antiseptics and disinfectants using the paper disc method to evaluate their effectiveness against the growth of specific organisms.

Materials
Nutrient agar plates, sterile swabs, broth cultures of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, sterile paper disc and antiseptics and disinfectants supplied by you. Each student should bring a sample of one anti-bacterial product to class.

Procedure
1. Using a sterile swab, heavily inoculate a nutrient agar plate with *Pseudomonas aeruginosa* (Figure 1). Label the plate your name, and P.a. Using a Sharpie, divide the plate into 3 or 4 sectors (depending upon how many trials we will be doing). In each sector record the name of the antiseptic / disinfectant being tested.
2. Repeat the above procedure in a new plate with *Staphylococcus aureus*. Figure 1. Method to swab an agar plate with bacterial medium.

Swab 1 - run across plate with an inoculated swab.
Swab 2 – run across the plate perpendicular to swab 1 with the same cotton swab, but without re-innoculating the swab.
3. Repeat the above procedure in a new plate with *Escherichia coli*.
4. Using sterile forceps, impregnate a sterile disc with the antiseptics or disinfectants you wish to evaluate and place the disc on the appropriate quadrant of the appropriate plate. Place the disc near the edge of the petri dish, but not touching it, in the middle of the quadrat.
5. Sterilize your forceps
6. Repeat step 4 for each petri dish for each microbe you are using, sterilizing the forceps after each paper disc is placed.
7. Repeat steps 4 – 6 for each antiseptic / disinfectant you are using.
8. Place the plates in the designated containers without inverting the plates.
Next lab period
9. Observe the plates that were exposed, and using a millimeter rule, measure the zone of inhibition that occurs around each disc. Measure from the center of the disc to the edge of the zone of inhibition.
10. Record your data and that of the other students to use in a lab write up dealing with the effectiveness of each type of antiseptic / disinfectant.