LABORATORY 1

Control of Microbes

OBJECTIVES

- To understand antiseptics and disinfectants and learn how to determine their effectiveness
- To understand the Kirby-Bauer or disc agar diffusion method to evaluate antibiotic sensitivity
- To understand the use of different types of heat treatments to control microbes

BACKGROUND

Control of microbes is important in the prevention of disease, both at home and in health care facilities. Control procedures can include good handling practices, use of antimicrobial chemicals, heating, and physical blocking of microbe transfer.

Chemicals are routinely used to inhibit or kill microbes, especially pathogens, in many settings. Those used on inanimate objects are referred to as disinfectants. These are, in general, harsher and more effective in microbistatic or microbicidal action than antiseptics. Antiseptics used on human or animal tissue are by necessity more gentle, in order to avoid damage to living tissue. These still must inhibit or eliminate pathogens effectively. The mode of action of many of these chemicals involves denaturing proteins or disrupting membranes. The effectiveness of a chemical agent against particular microbes is assessed in the laboratory prior to regular use. This may involve a disc diffusion plate assay, as used in this exercise.

Antibiotics are used to treat infectious disease. They have one of several modes of action for their inhibitory effect on bacterial cells, with minimal effects on human cells. Bacterial sensitivity to an antibiotic can vary with species and is often measured in the laboratory by the quick Kirby Bauer disc diffusion assay used here. For more precision, a more involved broth dilution method may be used to determine the minimum inhibitory concentration (MIC) or minimum bacteriocidal concentration (MBC) of an antibiotic or a chemical agent. These concentrations are important to know in determining proper dosage of the antimicrobial medication or chemical to be utilized.

Heat treatment is commonly used to kill microbes. This may include the use of dry heat (baking or incineration), boiling, or pressurized steam (autoclaving).

MATERIALS

2 nutrient agar plates
1 Mueller-Hinton agar plate
broth cultures of *E. coli, S. epidermidis, B. subtilis,* or *S. marcescens*
alcohol and spreader or sterile swabs
sterile filter disks
forceps
antiseptics and disinfectants (e.g., Listerine, bench disinfectant, Pine Sol, bleach)
antibiotic discs and dispenser
ruler (millimeters)
sterile test tubes

PROCEDURE 1: ANTISEPTIC AND DISINFECTANT EVALUATION

1. Label a nutrient agar plate for 3-4 separate quadrants.

2. Spread 1-2 drops of a bacterial culture on the nutrient agar plate. Partners could use different microbes—one gram-positive and one gram-negative, or use different compounds on the same microbe. The instructor will demonstrate the spread plate technique, which is another method to obtain a lawn of bacterial growth.

3. Pick up a sterile disc with flamed forceps and dip the edge of the disc into a beaker of chemical (do not immerse disc—it should not be sopping wet.) Place the disc in the center of a quadrant on the prepared plate. Press slightly on the disc with the forceps to secure it to the agar.

4. Repeat for other quadrants with other chemicals. Be sure to mark clearly what is in each section.

5. Incubate the plate at 37ºC for 1-2 days. Measure the diameter (in mm) of the zones of inhibition for each disc. It should be measured on the bottom of the plate, not the agar surface.

6. Compare your results with others. How effective was each product? Did it vary with bacterial strain? Were some results unexpected?

PROCEDURE 2: ANTIBIOTIC SENSITIVITY TESTING (KIRBY-BAUER ASSAY)

1. Prepare a spread plate of one bacterial culture on a Mueller Hinton agar plate.

2. Dispense antibiotic discs onto a prepared spread plate, using the disc dispenser. Be sure to record antibiotic codes and the disc potencies for those used.

3. Incubate the plates at 37ºC for 24 hours.

4. Measure and record the diameter (mm) of the zone of inhibition for each antibiotic. Using the Kirby Bauer zone interpretive chart, determine whether the microbe is susceptible or resistant to each antibiotic.

5. Class data will be compared to see differences between microbes.

PROCEDURE 3: HEAT SENSITIVITY OF MICROBES

1. Place a few drops of broth culture into a sterile test tube. Each group of students will do different microbes or different types of heat, as directed.

2. Treat the tube of bacteria with the appropriate heat treatment:
   boil 5 minutes in water bath
   heat in oven 10 minutes
   autoclave 15 minutes

3. Add 5 ml of nutrient broth to your tubes and incubate at 37°C for 24 hours. Be sure to include an unheated control for comparison of growth.

DISCUSSION

1. Would all antibiotics be expected to be equally effective against gram-positive and gram-negative bacteria? Explain.

2. What antibiotic might be useful to treat a staph infection?

3. What drug or type of drug is best used to treat an unidentified microbe? Why?

4. Why should one ideally standardize the amount of bacteria on the spread plate for these assays?

5. How did the different heat treatments compare for control of microbes? Was it different between strains?

KIRBY BAUER ZONE DIAMETER INTERPRETIVE DATA

Antimicrobial agent	Disc potency	Species	Resistant (mm)	Intermediate (mm)	Susceptible
Ampicillin (Am)	10 ug	Enterics Staphylococci Enterococcus Streptococc	<13 <28 <16 -	14-16 - - -	>17 >29 >17 >24
Bacitracin (B)	10 U	All	<8	9-12	>13
Cephalothin (CF)	30 ug	Enterics & Staph	<14	15-17	>18
Chloramphenicol (C)	30 ug	Enterics, Staph. & Enterococci S. pneumo Streptococci	<12 <20 <17	13-17 - 18-20	>18 >21 >21
Ciprofloxacin (CIP)	5 ug	Enterics, Staph & Enterococci	<15	16-20	>21
Erythromycin (E)	15 ug	Staph & Enterococci Streptococci	<13 <15	14-22 16-20	>23 >21
Novobiocin (NB)	30 ug	All	<14	15-16	>17
Penicillin (P)	10 U	Staphylococci Enterococcus Streptococci	<28 <14 -	- - -	>29 >15 >24
Rifampin (RA)	5 ug	Staph, Enterococcus & S. pneumo	<16	17-19	>20
Streptomycin (S)	10 ug	Enterics	<11	12-14	>15
Tetracycline (Te)	30 ug	Enterics & Staph Streptococci	<14 <18	15-18 19-22	>19 >23
Trimethoprim-Sulfamethoxazole (SXT)	1.25 ug 23.75 ug	Enterics & Staph S. pneumoniae	<10 <15	11-15 16-18	>16 >19
Vancomycin (Va)	30 ug	Staphylococci Entococcus Streptococci	- <14 -	- 15-16 -	>15 >17 >17

Adapted from BBL Sensidisc product insert.

KIRBY BAUER ZONE DATA

Organism	AM	B	CF	C	CIP	E	NB	P	RA	S	Te	SXT	Va