Lab Exercise #4 Home Edition
Control of Microorganisms: Student-designed Microbial Control Experiment

I. TERMINOLOGY:

Students should define and use the following terms:

agents of control	experimental control
Chemical agents of control	sensitive
Chemotheraputic agents of control	resistant
Disinfectant	Selective toxicity
Physical agents of control	Sterilize
Microbial Growth	Spectrum of activity for various antibiotics
Mode of action	Zone of inhibition

II. INTRODUCTION:

Control of microbial growth (inhibiting or killing microbes) is accomplished through physical, chemical and chemotherapeutic agents.

Physical Agents of Microbial Control

Physical agents of control include heat, freeze-drying, ultraviolet radiation and filtration. Heat denatures cellular proteins. Sterilization, the complete destruction of all viable cells, viruses and endospores, is accomplished by using the autoclave that combines the effects of heat and pressure. Autoclaved items are exposed to 121° C at 15 pounds per square inch (psi) of pressure for 15-20 minutes. Except for photosynthetic bacteria, most bacteria are harmed by ultraviolet radiation. Although sunlight contains the complete spectrum of short to long wavelengths of light, it is only the short invisible ultraviolet wavelengths that are injurious to these bacteria. Ultraviolet radiation is strongly absorbed by proteins and nucleic acids in cells; hence, the indications are that cellular damage involves changes in DNA. Depending on the dosage, ultraviolet radiation may cause enzyme inactivation, genetic mutation or death of the cell.

Chemical Agents of Microbial Control

Chemical agents of control like the disinfectants Lysol or Clorox destroy most vegetative cells and viruses. Chlorine is the active ingredient in Clorox**.** This is probably one of the most widely used disinfectants. It is used to disinfect water and for cleaning surfaces (e.g. floors, counters) and has proven effective in destroying HIV. The "killing" of microorganisms by chlorine and its compounds is due to the direct combination of chlorine with some cellular substances, thus 'poisoning' the cell. The mode of action of chlorine is generally considered to be the inhibition of enzyme activity and oxidization of cellular constituents to such an extent that they no longer perform normal metabolic functions. When chlorine reacts with organic materials, it is used up. Therefore, in order to be effective, chlorine concentrations must be high enough to allow the chlorine to attach to all the organic material present and still have some residual. Phenol is the active ingredient in Lysol. This disinfectant was first used by Lister in the mid 1800's to sterilize surgical instruments (aka: carbolic acid) There are many brand names and chemical cousins: cresols, hexylresorcinol, hexachorophene to name a few. Phenols exert their germicidal effect (i.e. mode of action) by denaturing proteins and destroying the selective permeability of the cell membrane (which permits "leakage" of cellular contents).

Limits of Physical and Chemical Microbial Control

Microbes respond differently to the affects of chemical and physical control agents. Endospores are very difficult to destroy. The autoclave is the most reliable way to destroy them. Some vegetative

cells are more difficult to destroy than others. Cells with mycolic acid, for instance, are more resistant to destruction than others. Viruses are generally easier to destroy than vegetative cells. Naked viruses are more difficult to destroy than enveloped viruses.

Both chemical and physical agents of control are not particularly stealth agents. The deleterious effects they exert on the microbe are a bit like bombing the target---everything (microbe, host cells, environment) is prone to the toxic effects of the agent that comes in contact with it. In contrast, chemotherapeutic agents are selectively toxic. They target some aspect of microbial metabolism (e.g. protein synthesis, cell wall production). Different antibiotics target different aspects of bacterial metabolism. The chemotherapeutic agent is an antimicrobial or antibiotic that should not harm the host cell.

Chemotherapeutic Agents of Microbial Control
Antimicrobics are drugs used to treat patients diagnosed with an infectious disease. Once the causative organism of a specific disease has been isolated, the physician needs to know, as soon as possible, which antibiotic is most effective in treating the disease. The laboratory uses the antibiotic sensitivity test to provide information regarding the effectiveness of various antibiotics to the physician. Disks are impregnated with the antibiotic. A nutrient agar plate is uniformly inoculated with bacteria and the disks are placed on the media. Over the incubation period, the antimicrobial diffuses in all directions out from the disk. If the microbe is sensitive to the specific antimicrobial in question, a zone of inhibited growth will occur around the antibiotic.

How to Test the Effectiveness of Microbial Control Agents
The basic principle for testing the effectiveness of an agent of control is as follows:

1. Expose the organism to the agent.
2. After a set amount of time, remove the agent.
3. Put the organisms in favorable growth media.
4. Look for reproduction/growth of organisms.
   Organisms sensitive to the agent will not grow; resistant organisms will continue to reproduce.

III. STUDENT-DESIGNED MICROBIAL CONTROL EXPERIMENT:

Each student will be designing an experiment to test the effectiveness of microbial control agents used in the home. This lab exercise is designed to get you thinking about the scientific method, and how researchers use it to discover verifiable truths.

Here are some general ideas to get you thinking about which experiment you might do, but the idea can come entirely from you as well. You want to make discoveries that could have an impact on your daily life and how you control microbial populations in your home environment and on personal possessions.

- Obtain sample from cell phone after a day's use, and after cleaning with some type of chemical, such as an antibacterial wipe. May want to utilize MacConkey's agar to see how much coliform bacteria is on your phone. (chemical control agent)
- Obtain a sample from food that has been kept in the fridge before and after cooking , once food has cooled (physical control agent)
- . Obtain a sample from your skin before and after using bacitracin. Mannitol salt agar would be a good choice for experiments in which you sample the skin. showering (physical control agent, water and soap reducing bacteria population on surface of your body(chemotherapeutic control).
- Obtain a sample from your dish washer when loaded with dirty dishes and after a wash cycle. MacConkey's would be a good choice to see if any coliform bacteria present in kitchen experiments. (physical control)
- Kitchen before and after cleaning with whatever agent you clean with. (chemical control)
- Baby / toddler toys before and after cleaning them. Both MAC and MSA agar would be interesting to use. (chemical control)

Remember, whatever experiment you choose to do, you always need to have an **experimental control**. When taking a sample before and after your experimental treatment, the *before* sample is the control. The *after* sample is the experimental one.

Be sure to test only one **variable**; only one thing that varies between your control and experimental sample. For example if testing the effectiveness of an antimicrobial wipe on reducing the microbial population on your cell phone, cleaning the phone with the wipe would be your experimental variable. All other possible variables would be control variables, things that you aren't altering, for example, how often you wash your hands, how much time you spend on the phone, where you usually keep your phone. If you test more than one experimental variable at the same time, that makes it impossible to know which had an effect, and how much of an effect.

For this laboratory exercise, it is very important for you to remember that the word **control** is being used in two different contexts. **Microbial control** means *killing microbes*. When referring to **experimental design**, the word control *refers to the sample that you will obtain prior to your experimental treatment*.

Materials

Available Supplies

Plates of TSY agar
Plates of MAC agar
Plates of MSA agar
Sterile Swabs
A microbial control agent that you use in your home

You need to determine the specific materials that you will require to conduct your microbial control experiment. To know what you need, you will first have to design your experiment.

First Microbial Control Assignment

You will need to develop the procedure for your microbial control experiment. Write out a very precise, detail-filled outline indicating exactly what you plan to do. In your outline specifically indicate which type of microbial control your experimental treatment represents (physical, chemical, chemotherapeutic), and describe its specific mode of action - specifically how it disables or destroys microbes on a cellular level. Also name your experimental variable. You will be able to use this information again to write the introduction to your Lab Report. Please present this outline in a very well-organized way. In other words, don't make me search for your information.

There will be an UPLOAD for your completed Experimental Outline, listing due date, in Moodle. I will give you feedback on your experimental design. If you turn in an assignment that is very sparse and does not appear to have been well thought out, that is the only feedback you will get.

Second Microbial Control Assignment

You will be doing a lab report to describe your experiment and its results. This will not be in question-and-answer format, like the pervious lab report. This will be an actual lab report you will write. Utilize the Lab Report #4 Form as the starting point of the lab report that you will create.

For basic instructions on how to write your lab report, see the Lab Report Template. Also carefully read *How to Write a Paper in Scientific Journal Style and Format*, from Bates College. Using these two resources, in addition to the instructions on this page, you should be able to produce a well-organized, well thought out paper.

For instructions on how to cite references, see Siting References in Scientific Research Papers.

There will be an UPLOAD for your completed Lab Report, listing the due date, in Moodle.