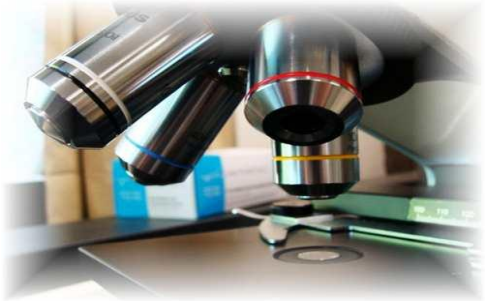

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• The SPO Virtual Classrooms offer many educational resources, including practice test questions, review questions, lecture PowerPoints, video tutorials, sample assignments and course syllabi. New materials are continually being developed, so check back frequently, or follow us on Facebook (Science Prof Online) or Twitter (ScienceProfSPO) for updates.

• Many SPO PowerPoints are available in a variety of formats, such as fully editable PowerPoint files, as well as uneditable versions in smaller file sizes, such as PowerPoint Shows and Portable Document Format (.pdf), for ease of printing.

• Images used on this resource, and on the SPO website are, wherever possible, credited and linked to their source. Any words underlined and appearing in blue are links that can be clicked on for more information. PowerPoints must be viewed in *slide show mode* to use the hyperlinks directly.

• Several helpful links to fun and interactive learning tools are included throughout the PPT and on the Smart Links slide, near the end of each presentation. You must be in *slide show mode* to utilize hyperlinks and animations.

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Image: Compound microscope objectives, T. Port

Laboratory Exercise 4

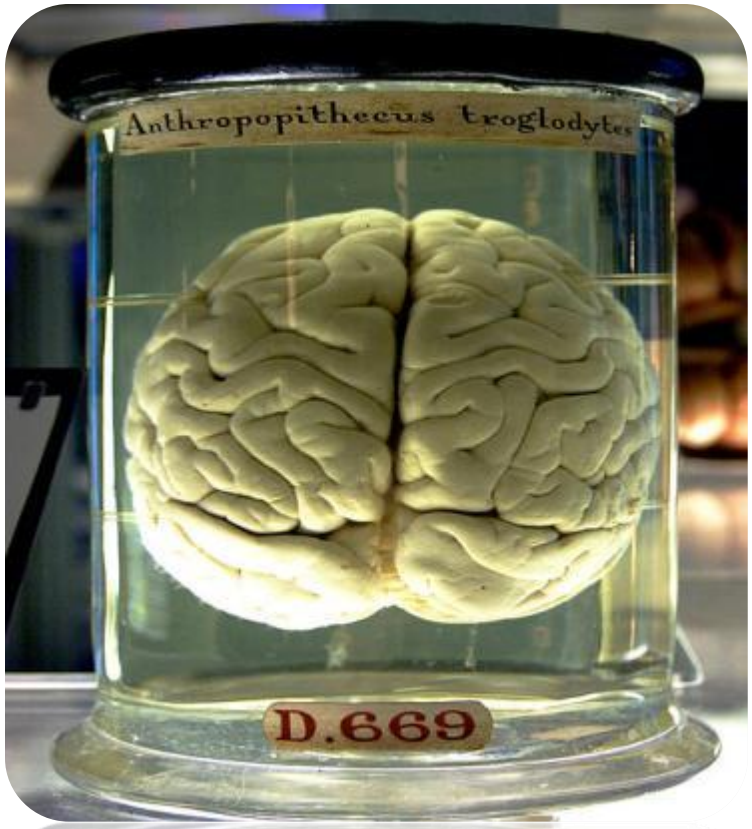
Microbial Control:

Physical, Chemical & Chemotherapeutic

Image: Zones of bacterial inhibition from antibiotic disks, T. Port

What am I going to learn from Lab Topic #4?
Microbial Control

- Investigate the effectiveness various agents of control.
- Assess the effectiveness of heat, and of chemical disinfectants, in killing vegetative cells versus endospores.
- Evaluate ultraviolet radiation as a mechanism of control.
- Examine the fundamentals of antibiotic sensitivity testing.

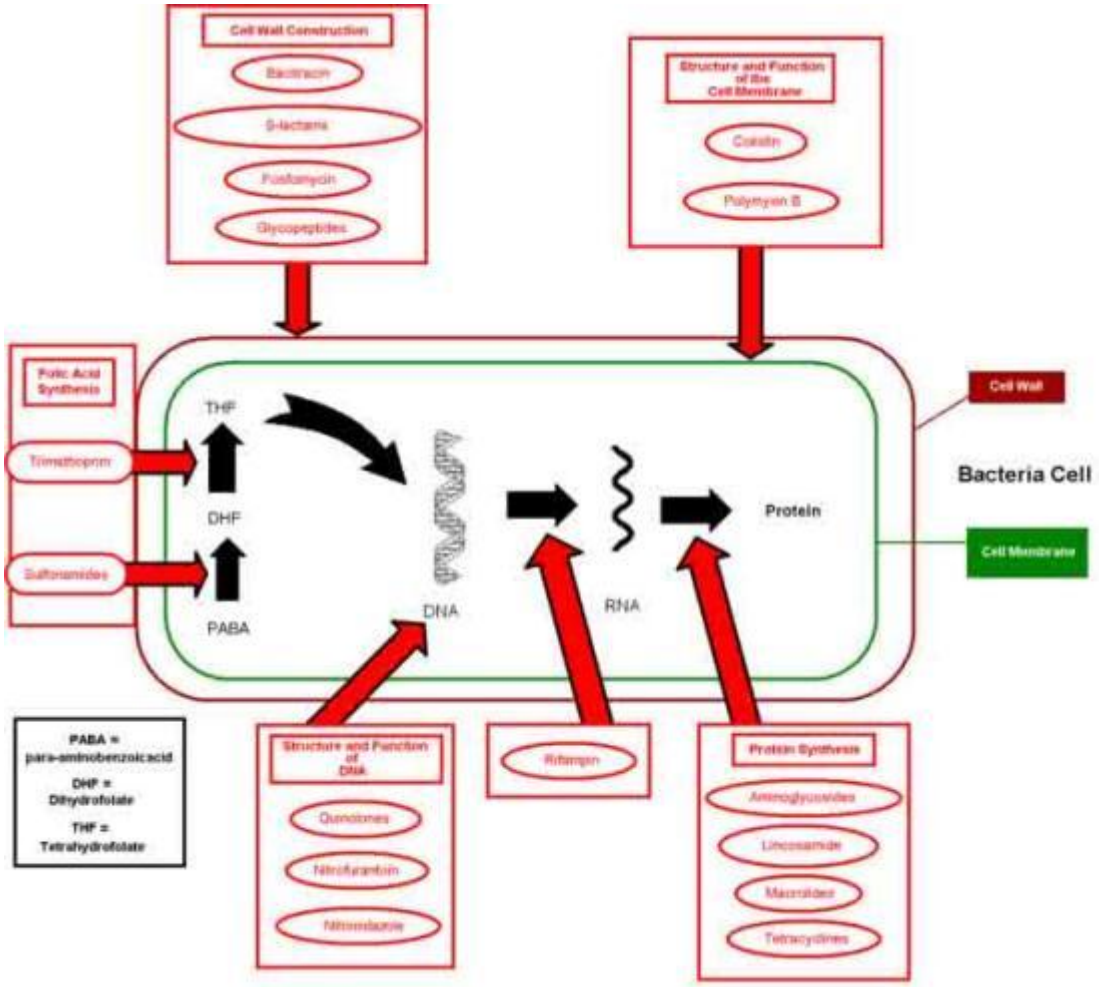


Please plug in your microincinerators.

Image: Chimp brain in a jar, Gaetan Lee

Action of Antimicrobial Agents

- Many types of chemical and physical microbial controls
- Modes of action fall into two basic categories:
  1. Alteration of bacterial cell walls or cytoplasmic membranes
  2. Interference with protein and nucleic acid structure



**** You will be using 4 TSY plates in today's lab. Let's pour them now. ****

Image: Antibiotic Modes of Action, J. Raghu

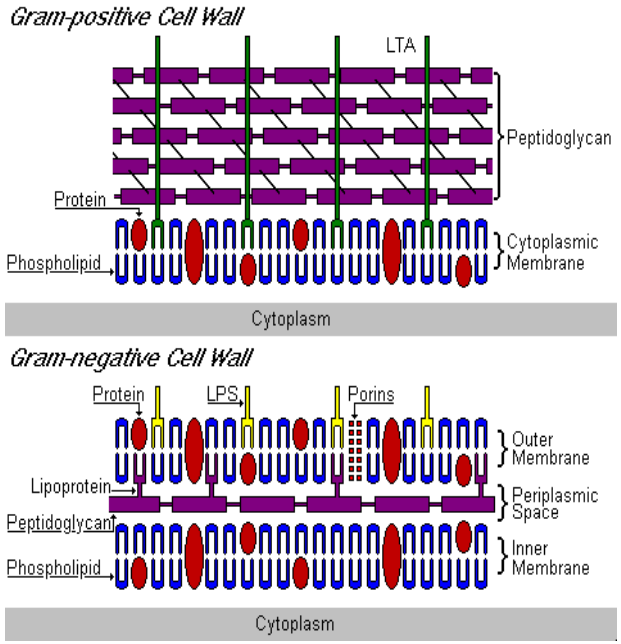
1. Alteration of **Cell Walls & Membranes**

- **Cell wall** maintains integrity of cell.
  - When disrupted, cannot prevent cell from bursting due to osmotic effects.

- **Cytoplasmic membrane** contains cytoplasm and controls passage of chemicals into and out of cell.
  - When damaged, cellular contents leak out.

- **Viral envelope** responsible for attachment of virus to target cell.
  - Damage to envelope interrupts viral replication.

  - Nonenveloped viruses have greater tolerance of harsh conditions.



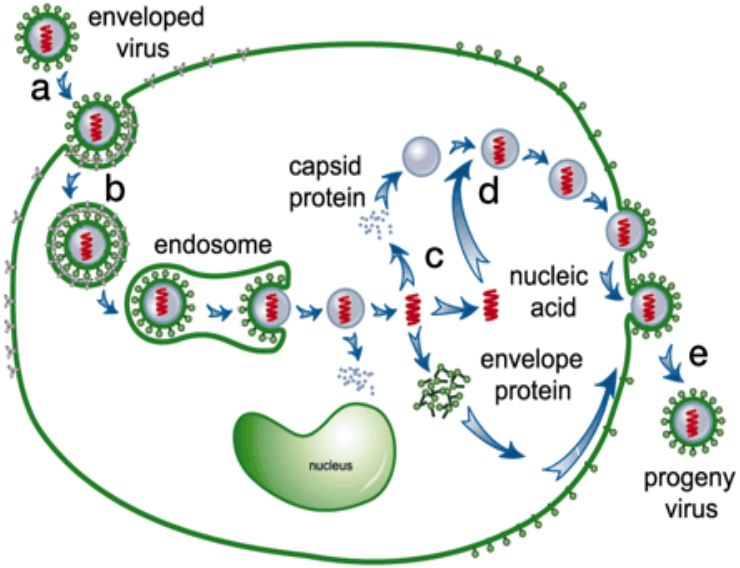
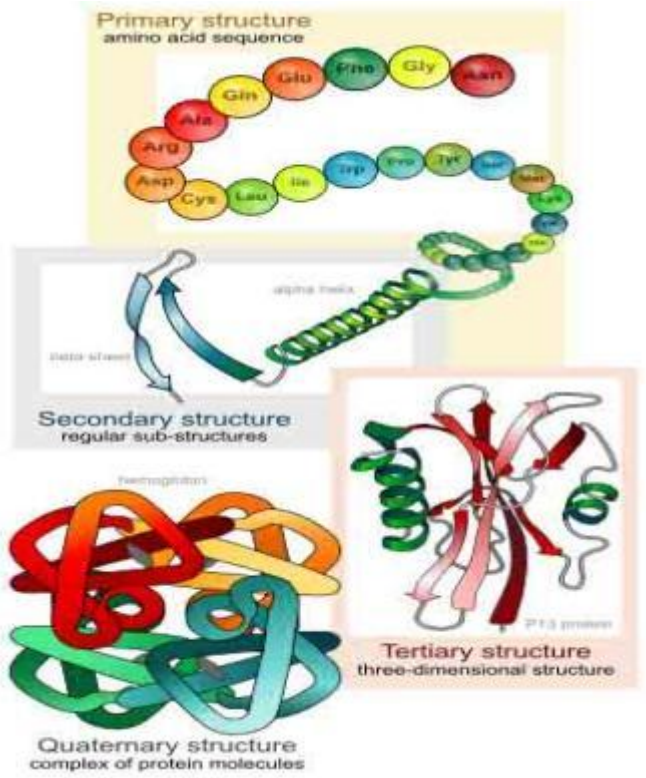


Image: Viral life cycle, National Academy of Sciences

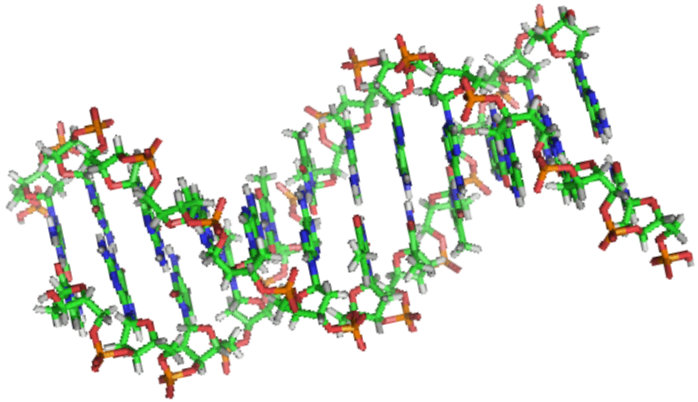
From the Virtual Microbiology Classroom on ScienceProfOnline.com

2. Interference with **Proteins** & **Nucleic Acid** Structure



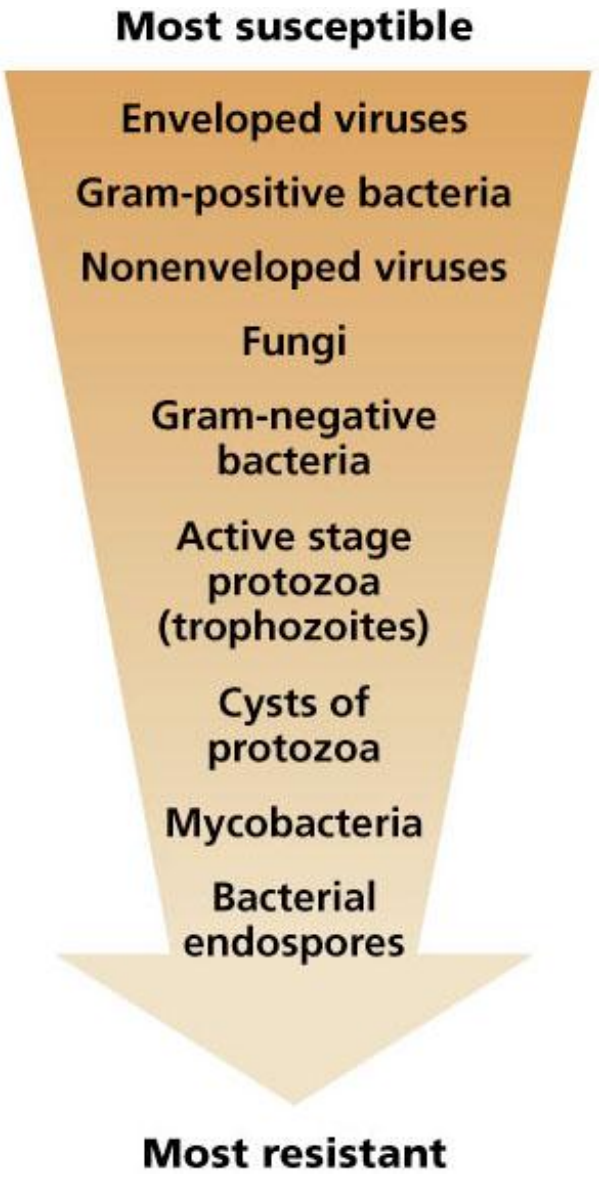
- **Protein** function depends on 3-D shape.
  - Extreme heat or certain chemicals denature proteins.

- **Nucleic Acids** can be damaged or destroyed by chemicals, radiation, and heat.
  - Can produce fatal mutations.
  - Can halt protein synthesis through action on RNA.



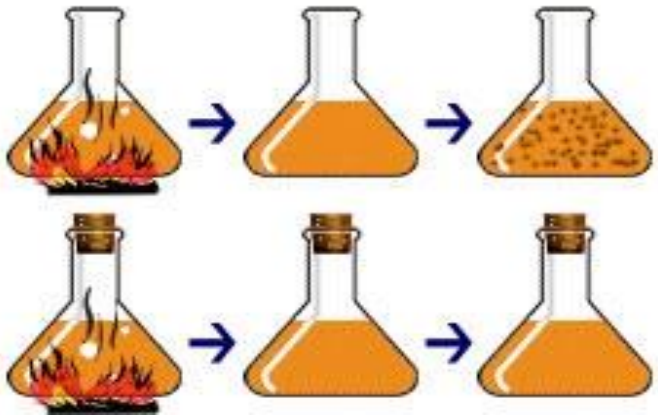
Images: Levels of protein structure, M Ruiz, DNA, Richard Wheeler

Relative Susceptibility of Microorganisms



Protocol for Testing a Control Agent

The basic principle for testing any control agent, (whether temperature, chemical or antibiotics) is always the same:



1. Expose the organism to the agent.
2. Remove the agent.
3. Put organisms in favorable growth medium.
4. Look for reproduction of organisms.

Methods of Microbial Control That We Will Be Examining

- **Physical**
  - Heat ←
  - UV
- **Chemotherapeutic**
  - Antimicrobic drugs, like antibiotics
- **Chemical**
  - Bleach, other chemical disinfectants

Effects of High Temperatures

- Denaturation of proteins
- Interference with integrity of cytoplasmic membrane and cell walls
- Disruption of structure and function of nucleic acids

Boiling

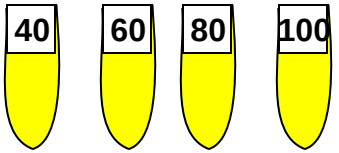
- Kills vegetative cells of bacteria, protozoa and fungi, and most viruses in 10 minutes (at sea level).
- Boiling time is critical.
- Some heat is lost as steam.
- Endospores, protozoan cysts, and some viruses can survive boiling

Autoclaving

- Pressure applied to boiling water prevents steam from escaping.
- Boiling temperature increases as pressure increases.
- Autoclave conditions: 121°C (~250°F), 15 psi, 15 minutes.

Image: Autoclave, Astell Scientific

A: Microbial Control Using Heat



- Each pair of lab partners should inoculate 4 tubes of nutrient broth with *E. coli* and 4 tubes with *Bacillus* species (spp.).
- Label the tubes clearly indicating which microbe they contain and include your initials.
- You will be exposing each tube of *E. coli* and each tube of *Bacillus* to different temperatures.
- Label each of the 4 *E. coli* tubes with the temperature it will be exposed to: 40°C, 60°C, 80°C, and 100°C.
- Label each of the 4 *Bacillus* tubes with the temperature it will be exposed to: 40°C, 60°C, 80°C, and 100°C.
- Place each tube into the appropriate water bath for 10 minutes.
- Remove the tubes after 10 minutes at temperature. Put them into your "SAVE" test tube rack.

How to Inoculate a Broth Medium

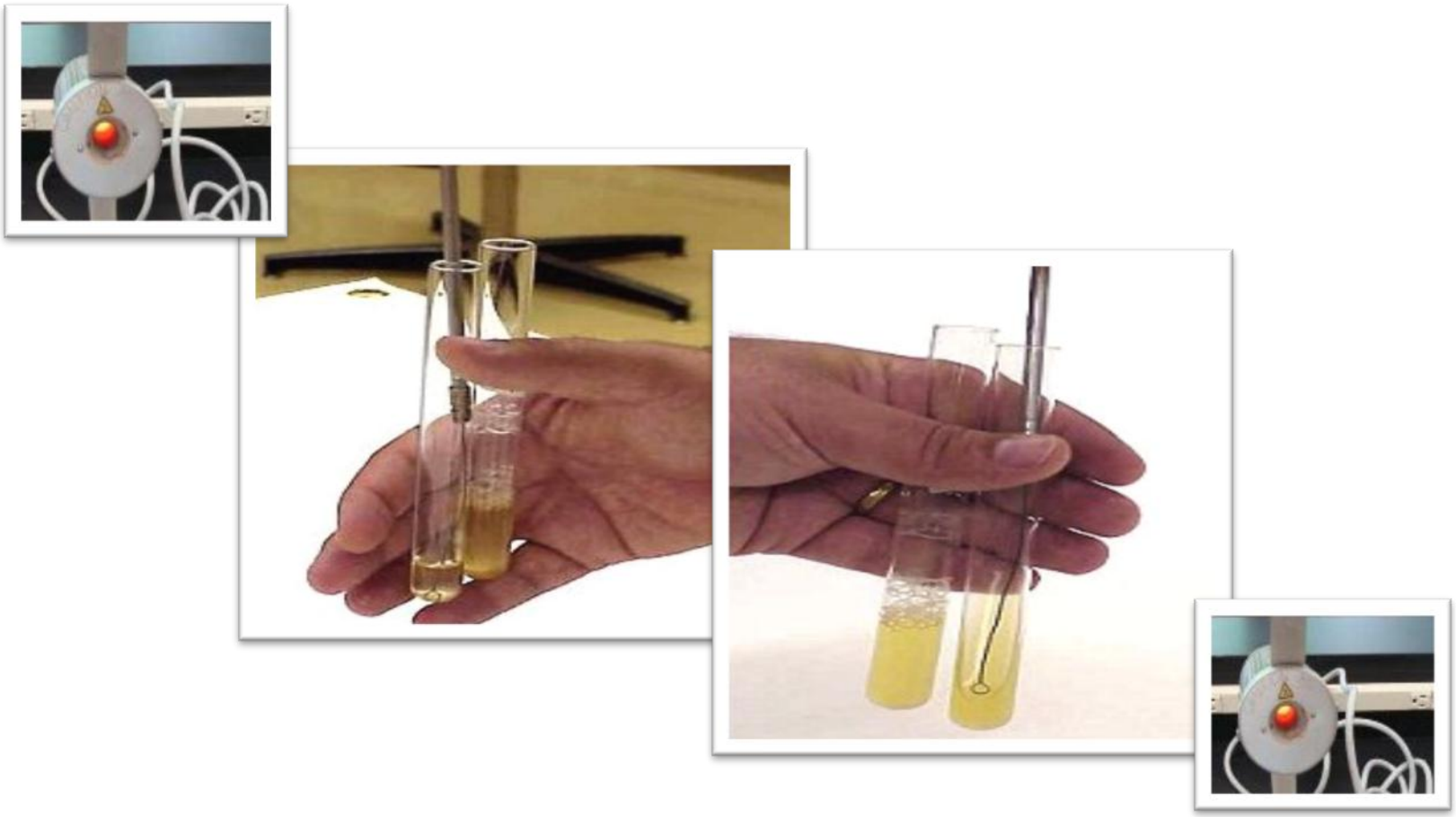


Image: Microincinerator, T. Port; Test tubes, Source unknown

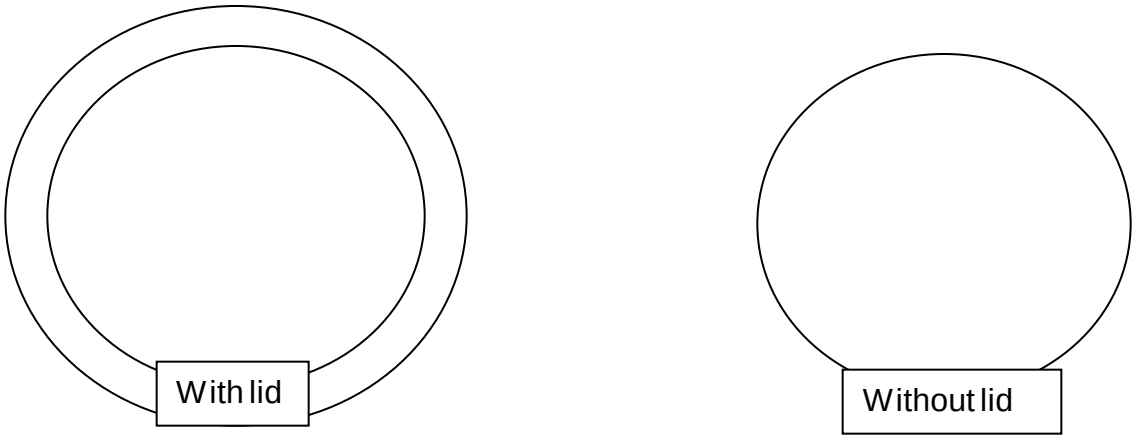
Methods of Microbial Control That We Will Be Examining

- **Physical**
  - Heat
  - **Ultra Violet Radiation** ←
    - Sunlight contains the complete spectrum of short to long wavelengths of light.
    - The short, invisible ultraviolet (UV) wavelengths are injurious to non-photosynthetic bacteria.
    - UV radiation is strongly absorbed by proteins and nucleic acids.
    - UV radiation may cause enzyme inactivation, genetic mutation or death.
- **Chemotherapeutic**
- **Chemical**

Image: Sun's corona as seen in deep ultraviolet by the Extreme ultraviolet Imaging Telescope, NASA

B. Effectiveness of UV Radiation as a Way to Control Bacteria

- Use 2 TSY Plates.
- Inoculate each by dipping a sterile swab in the culture tube of *E coli* and then wiping the swab over the **entire surface** of the agar.
- Expose both UV radiation for 5 minutes in the UV box.



Methods of Microbial Control That We Will Be Examining

- **Physical**
  - Heat
  - Ultra Violet Radiation
- **Chemotherapeutic** ←
  - Antimicrobic drugs, like antibiotics
- **Chemical**

C. Effect of Antimicrobials

- **Antimicrobics** are drugs used in the treatment of infectious disease.
- **Sensitivity disks** can show us which antimicrobic will be most effective in controlling an organism.
- The disks are impregnated with the **antibiotic**.
- A nutrient agar plate is uniformly inoculated with bacteria and the disks are placed on the media.
- You will paint one TSY plate with Staph epi and one with E. coli, and then expose both dishes to the same types of antibiotics.
- ***Q:*** *Why are we using Staph vs.* ***E. coli?***
- 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 inhibition** (an area without bacterial growth) will occur around the antibiotic.

Image: Zones of bacterial inhibition from antibiotic disks, T. Port

Methods of Microbial Control That We Will Be Examining

- **Physical**
  - Heat
  - UV
- **Chemotherapeutic**
  - Antimicrobic drugs, like antibiotics
- **Chemical** ←
  - Bleach & other chemical disinfectants

Chemical Microbial Control

Chlorine vs. Lysol

- The purpose of this part of lab is to compare the effectiveness of chlorine and Lysol in killing bacterial vegetative cells and destroying endospores.
- We will compare both the agent and the strength .

Image: Lysol on shelf, Bnilsen

Chlorine – Mode of Action

- Used to disinfect water and for cleaning surfaces (e.g. floors, counters) and has proven effective in destroying HIV.
- Kills microbes by inhibiting enzyme activity and oxidizing cellular contents so that they no longer perform normal metabolic functions.
- Chlorine reacts with organic materials in the cell and is used up.
- To be effective, chlorine concentrations must be high enough to allow chlorine to attach to all the organic material present and still have some residual.

Lysol *(Phenol)* – Mode of Action

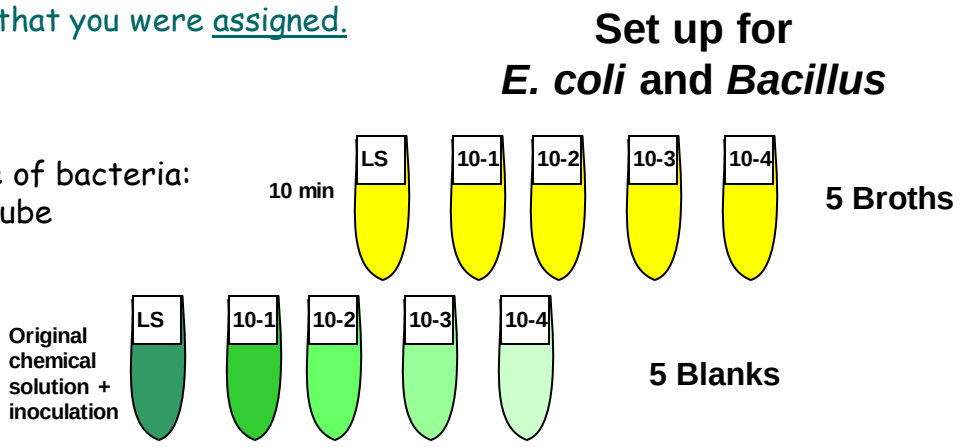
- First used by Lister in the mid 1800's to sterilize surgical instruments (aka: carbolic acid).
- Phenols exert their germicidal effect by denaturing proteins and destroying the selective permeability of the cell membrane (which makes cells leak).

Image: Lysol on shelf, Bnilsen

D. Chemical Microbial Control

1. Materials:
   a. The chemical agent and type of bacteria that you were assigned.
   b. 10 tubes of broth
   c. 10 blank (empty) tubes

2. Label 1 blank and 1 broth tubes with dilution & type of bacteria:
   a. label strength (LS) = one blank and two broth tube
   b. 10-1 = one blank and one broth tube
   c. 10-2 = one blank and one broth tube
   d. 10-3 = one blank and one broth tube
   e. 10-4 = one blank and one broth tube



3. Working with the blank tubes, pipet 10 ml of each of the agent dilutions (i.e. label strength, 10-1, 10-2…) into the appropriately labeled empty tube.

4. Note the time, then inoculate the five corresponding blank tubes that now *contain* the various dilutions with one loopful of *E. coli or Bacillus.*

5. **After 10 minutes**, aseptically transfer one loopfull from the agent dilution tube labeled "label strength" to a culture broth tube that is appropriately labeled.

6. Repeat this step for each of the dilutions. *Make sure that you are sterilizing your loop between each transfer!!*

7. Place the serial dilution tubes (the "blanks") in the DISCARD rack on the side bench.

9. Place the inoculated broth tubes in the "SAVE" labeled green bin for incubation and storage until next week when we will determine the patterns of growth.

Confused?

Here are links to fun resources that further explain microbial control:

Smart Links

- **Microbial Control Laboratory** Main Page on the Virtual Microbiology Classroom of Science Prof Online.
- Control of Microbial Growth, Todar's Online Textbook of Bacteriology.
- Play Pandemic 2 a video game of strategy, where you try to become a successful pandemic microbe and infect the world. My 14-year old, crazy-smart daughter recommends this one to you.
- Play Disease Defenders educational video game, Rice University.
- Pasteur's Experiment in which he used heat to control microbes animation and quiz from WH Freeman.

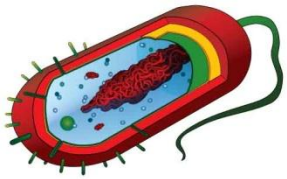
(You must be in PPT slideshow view to click on links.)

Are microbes intimidating you?

Do yourself a favor. Use the…

Virtual Microbiology Classroom *(VMC)* !

The VMC is full of resources to help you succeed, including:



- practice test questions
- review questions
- study guides and learning objectives

You can access the VMC by going to the Science Prof Online website
www.ScienceProfOnline.com

Images: E. coli, Giant Microbes; Prokaryotic cell, Mariana Ruiz