SACRAMENTO STATE
Leadership begins here.

Chapter 12:
Sterilization & Disinfection
Chapter 6:
Counting bacterial growth

Lecture Exam #1 is one week from today.
Bring a Scantron 882 form!

Fall 2006
Lectures: MW Noon
Office Hours: Mondays & Wednesdays
9:00-10:00 AM

Microorganisms are everywhere

Occasionally, this is a problem:

- Food spoilage
- Infectious disease transmission
- Research / diagnostics

Solution:

Sterilization
or
Disinfection

★Sterilization

- Killing or removal of **all** microorganisms in a material or on an object
- Including spores & endospores

★ Disinfection

- **Reducing** the number of pathogenic microorganisms
- Sterility is NOT the goal

Different microbes have different susceptibilities to antimicrobial agents

Staphylococcus aureus (Gram +) *Escherichia coli* (Gram -)
Filter paper disks soaked in various disinfectants

Sterilization: **Dry Heat**

Dry Heat (hot air oven; gas flame):

- Metal objects
- Glassware
- Oils & powders (that can't get wet)

Example: 171°C for 1 hour, depending on volume (as dry heat penetrates slowly)

- If lower temperature, must increase time to achieve sterility

Sterilization: **Autoclaving**

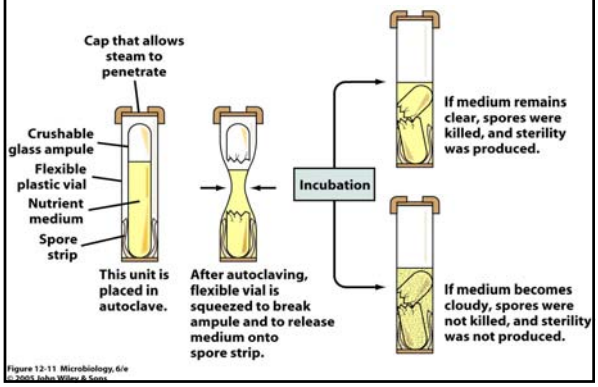
Moist Heat (**autoclaving**) ★

- Pressurized steam, above the boiling point of water

Example: 121°C, 15 lb/in^2 for 15 minutes

- Air must be removed so the chamber fills with steam
- Steam must penetrate the objects to be sterilized
  - Items should not be sealed, wrapped, or too crowded; otherwise autoclave time must be increased

Autoclave performance must be monitored



Sterilization: **Ethylene oxide**

- Flammable gas ★
- Mechanism: Alkylating agent
  - Disrupts function of proteins, nucleic acids
    - This kills microbes, including viruses
  - Carcinogenic (causes cancer)
  - Works at room temperature
- Used to *sterilize heat- or moisture-sensitive materials*
  - Plastics, rubber, etc.
  - Very important for medical equipment

Sterilization: **Radiation**

- **Ionizing radiation** is used to sterilize medical equipment
  - Ionizing radiation can also be used to preserve foods, though this process has met with resistance in the U.S.; some people think "irradiated" means "radioactive"
    - » See news article for more details

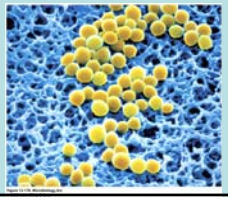
Sterilization: **Filtration** ★

- The passage of a material through a filter, or straining device
- Requires filters with *very small* pores
- Often used to sterilize solutions of heat-sensitive compounds (e.g., drugs)

A vacuum pulls solution through the filter, into a sterile bottle.

Sterilization: Filtration

- Filters are made with different pore sizes, depending on whether you need to filter out viruses
  - Viruses are MUCH smaller than bacteria



Staphylococcus epidermidis cells trapped on a 0.22 μm filter

A 0.025 μm filter would also trap viruses

★Disinfection: **HEPA Filtration**

- High-efficiency particulate air filters (HEPA) ★
  - are **used in ventilation systems** where microbial control is important
    - High-rise buildings, airplanes, operating rooms, burn units, laboratory hoods, hospital rooms of patients with highly contagious disease (esp. tuberculosis / TB)

Disinfection: **Pasteurization**

- Invented by Louis Pasteur to prevent souring of wine
- Does NOT sterilize ★
- DOES kill important pathogens likely to be found in milk
  - *Salmonella, Mycobacterium, Listeria*
- Temperature is kept low enough so milk still tastes OK
  - 71.6 ºC for 15 seconds; or,
  - 62.9 ºC for 30 minutes

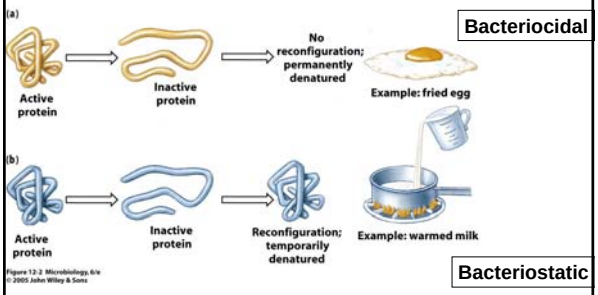
Disinfection: Various chemical agents

- A wide variety of chemicals can kill, or inhibit the growth of, microorganisms
- *Some* are safe to use on skin; these are usually called antiseptics

Chemical Disinfection: Mechanisms

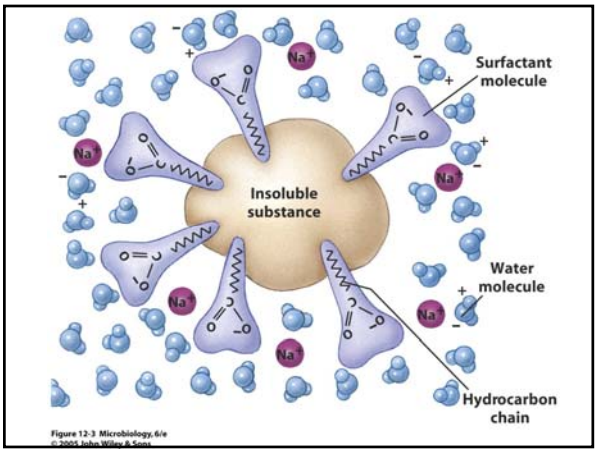
1. Protein modifications
   - **Denaturation**: hydrogen & disulfide bonds broken
     - » May be reversible
   - **Hydrolysis**: cleavage of proteins into amino acids
   - **Addition of chemical groups** (halogens, alkyl groups, etc.)

Protein denaturation



Chemical Disinfection: Mechanisms

2. Lipid dissolution: Surfactants
   - Surfactants reduce the surface tension of a liquid
   - Often consist of a charged end & hydrocarbon tail
   - Surfactants dissolve lipids and wash them away
   - Surfactants affect membranes
- Soaps & detergents; alcohols; quaternary ammonium compounds (lab bench cleanser)



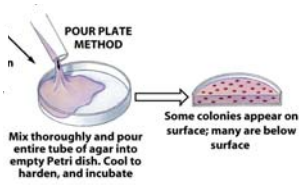
Soap: how does it work?

- Solubilizes lipids, helps to wash away microbes in the rinse water
  - Vigorous scrubbing helps a lot
- Alkalinity + sodium: kills some microbes

Measuring bacterial growth: Standard Plate Counts

You have a broth culture and you want to know how many bacteria are in it.

Can take 1 mL of broth culture, add it to 9 mL cool but still melted agar, mix and pour into a Petri plate.

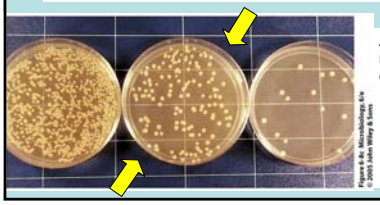


Wherever a viable cell lands, it will form a colony.
(colonies on the agar surface will be larger than colonies inside the agar)

 # colonies = # living bacteria per mL

★ also called *colony forming units* **(CFU)**

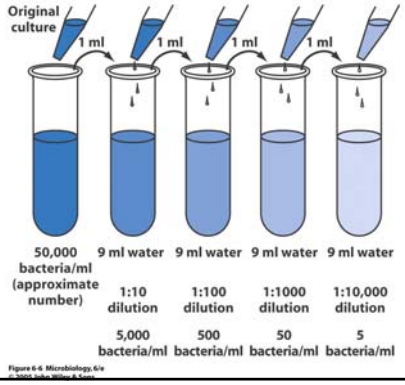
as not all the bacteria in the culture may be able to grow into colonies, and therefore are not counted.

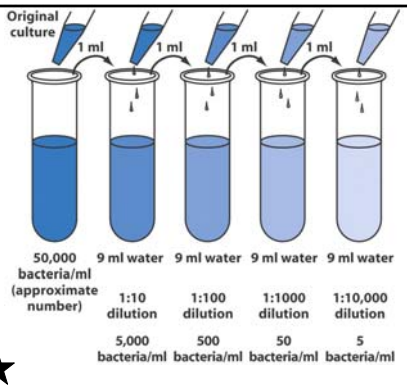


A reasonably countable number of colonies is ★ considered 30-300 per plate.

What if you have *many more* than that?

Serial Dilutions

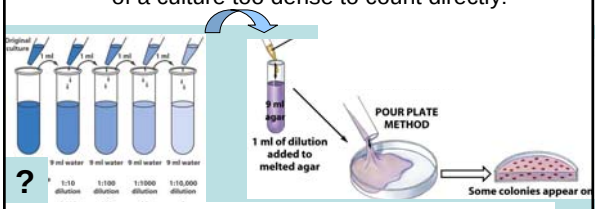




★

Diluted cell count is:	10^{-1}	10^{-2}	10^{-3}	10^{-4}	of original culture
"Dilution factor" is:	10	100	1000	10,000	

Use serial dilutions to calculate viable cell concentration of a culture too dense to count directly:



Count colonies on plate = 150

Calculate # of CFU / mL in original, undiluted culture:

★ **CFU (colonies) x Dilution factor**

$150 \times 10^4 = 1.5 \times 10^6$
(1,500,000 per mL)

Bacterial growth: Turbidity

More bacteria in broth = more turbid (cloudy)

Measure turbidity with ***spectrophotometer***

- Spectrophotometer detects how much light of a particular wavelength penetrates a solution
- The amount of light (600 nm) absorbed correlates with the concentration of bacteria in the culture
- **Accurate only within a range**: doesn't work if too few bacteria/mL or too many ★
- Also **cannot discriminate between living and dead cells** (unlike CFU from plate count)

Bacterial Growth: Calculating Generations per hour (**k**)

$$\mathbf{k} = \frac{\log N_t - \log N_0}{0.301 * (\text{elapsed time in hours})}$$

{0.301 = log 2}

{N_t = # bacteria per mL at time t;
N_0 = # bacteria per mL at time zero}

Generation time (in hours) = 1/**k**

You do NOT need to memorize this equation, but you should be able to use it.

Relevant reading in Black's Microbiology:

(pages from 6th edition)

- Ch. 12: bits from throughout the chapter were discussed
- Ch. 6: p. 145-147; p. 150

- Over the next few weeks for lab, review ch. 6 p.160-167 (on culturing bacteria)