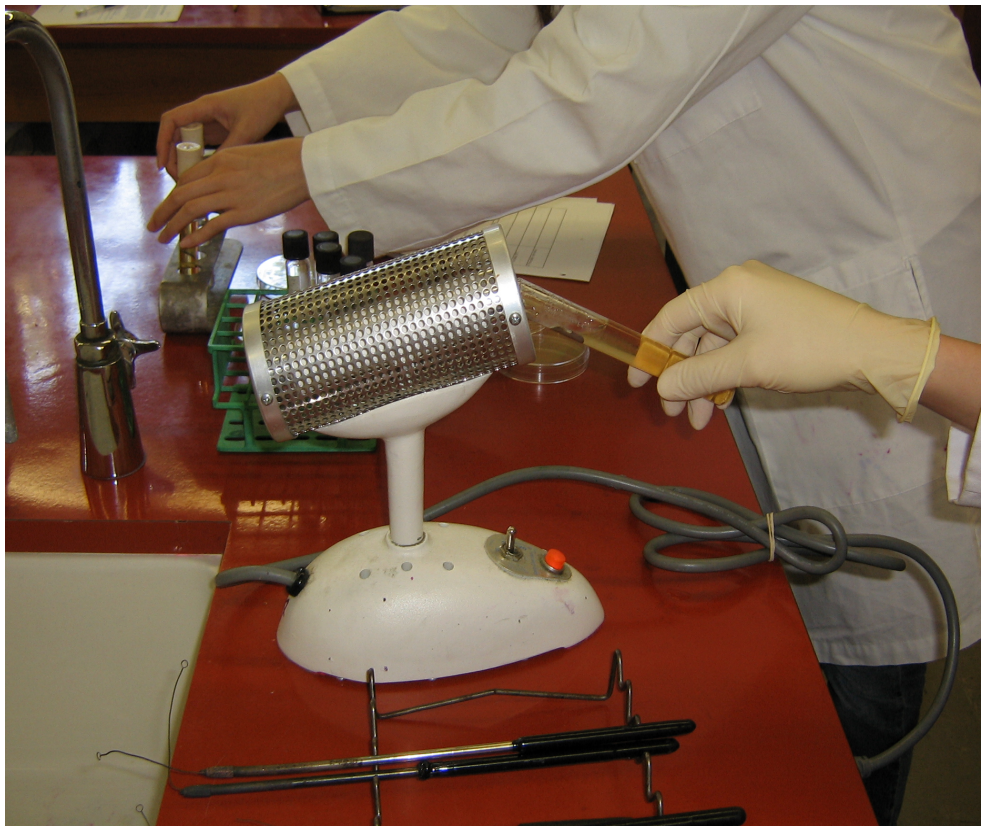
Unit 3

Control!

III. CONTROL OF MICROORGANISMS: Some definitions

- Sepsis: Greek for decay or putrid: bacterial contamination as in *"septic tank"*
- Asepsis: (a) without infection; (b) free of infectious agents
  - Aseptic techniques in our lab



More Definitions to know! (table

Table 7.1 Terminology Relating to the Control of Microbial Growth

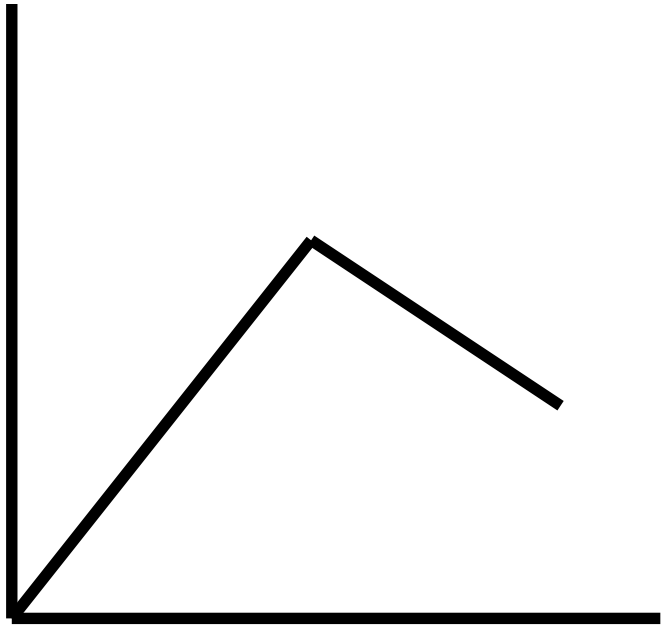
	Definition	Comments
Sterilization	Destruction or removal of all forms of microbial life, including endospores but with the possible exception of prions.	Usually done by steam under pressure or a sterilizing gas, such as ethylene oxide.
Commercial Sterilization	Sufficient heat treatment to kill endospores of *Clostridium botulinum* in canned food.	More-resistant endospores of thermophilic bacteria may survive, but they will not germinate and grow under normal storage conditions.
Disinfection	Destruction of vegetative pathogens.	May make use of physical or chemical methods.
Antisepsis	Destruction of vegetative pathogens on living tissue.	Treatment is almost always by chemical antimicrobials.
Degerming	Removal of microbes from a limited area, such as the skin around an injection site.	Mostly a mechanical removal by an alcohol-soaked swab.
Sanitization	Treatment intended to lower microbial counts on eating and drinking utensils to safe public health levels.	May be done with high-temperature washing or by dipping into a chemical disinfectant.

Make sure you know these terms and definitons!

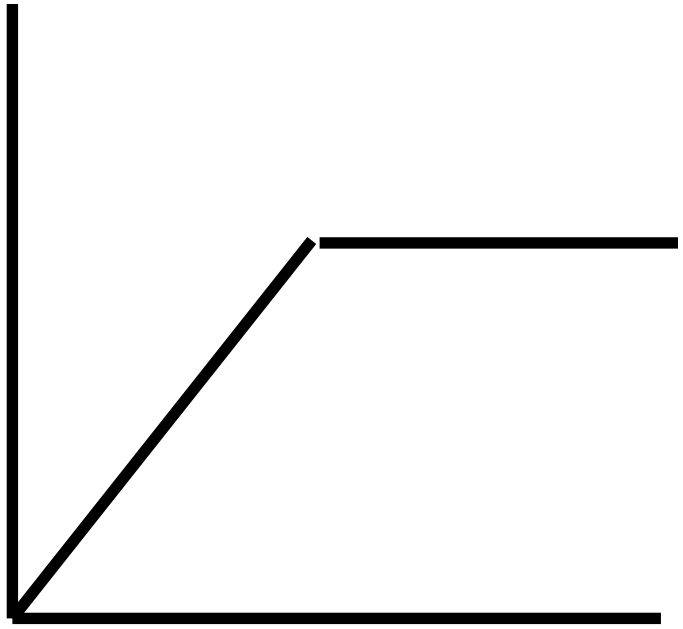
Cidal versus static action

- -cide or -cidal refers to killing (germicide, tuberculocidal, fungicide etc)
- -stasis or –static or stat refers to inhibition without killing (fungistatic; bacteriostatic, etc)
- many important control methods are static rather than cidal

Cidal vs Static action



cidal



static

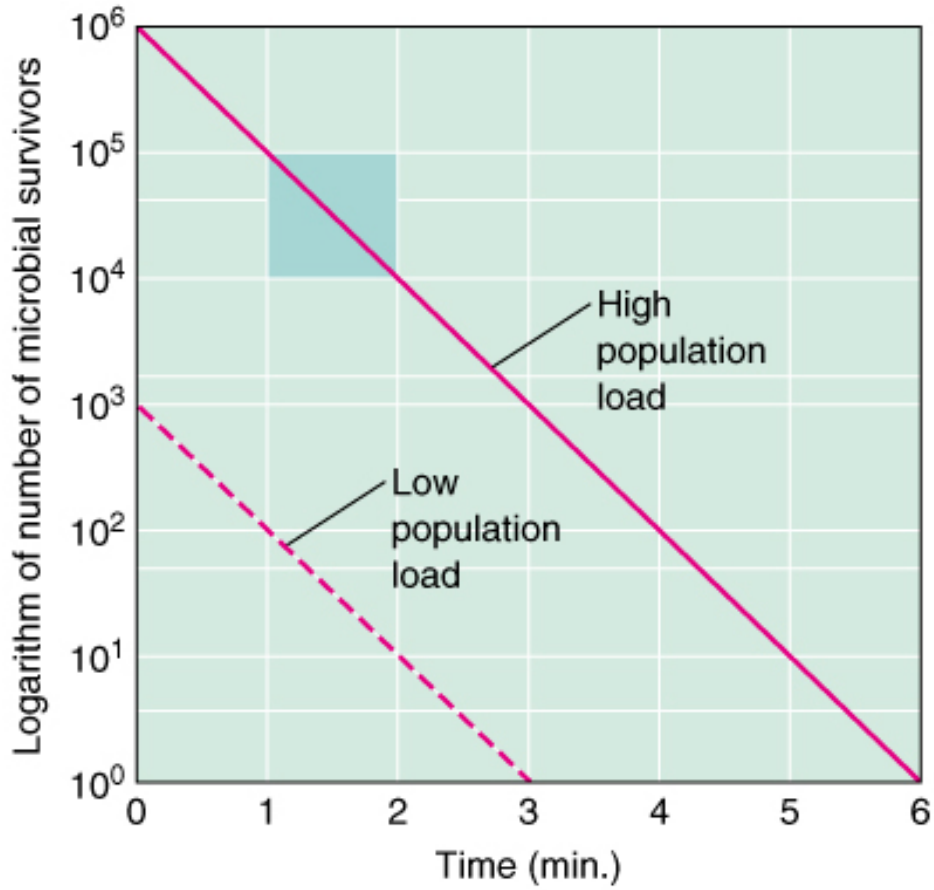
Logarithmic death rate

- upon exposure to a lethal agent organisms DO NOT all die at the same time; they die at a logarithmic rate
- the same percentage of survivors dies during each equal time period
- sterility is achieved when number of survivors is less than one (could not have 0.2 survivor)

Table 7.2 Microbial Exponential Death Rate: An Example

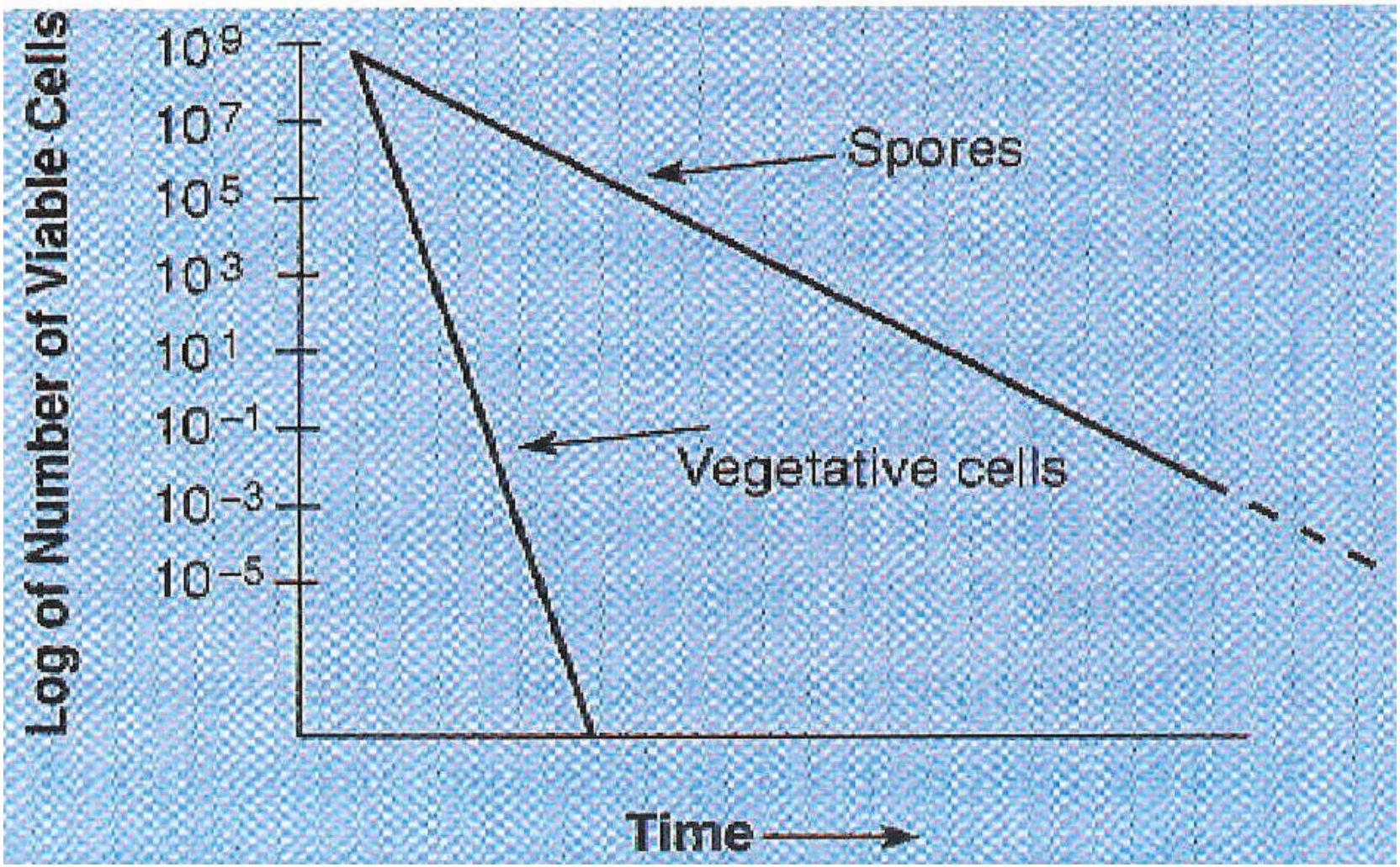
Time (min)	Deaths per Minute	Number of Survivors
0	0	1,000,000
1	900,000	100,000
2	90,000	10,000
3	9000	1000
4	900	100
5	90	10
6	9	1

- effect of degree of contamination
- (contamination = load)



(b)

Effect of spore formation on death rate.



- Good Essay Question!: Explain the difference between cidal and static. Explain cell death as a function of time, degree of contamination, and organism type (vegetative cells vs. spores).

Control Methods: Physical vs Chemical

- First physical control method (heat):

Table 7.5 Physical Methods Used to Control Microbial Growth

Methods	Mechanism of Action	Comment	Preferred Use
Heat			
1. Moist heat			
a. Boiling or flowing steam	Protein denaturation	Kills vegetative bacterial and fungal pathogens and almost all viruses within 10 min; less effective on endospores	Dishes, basins, pitchers, various equipment
b. Autoclaving	Protein denaturation	Very effective method of sterilization; at about 15 psi of pressure (121°C), all vegetative cells and their endospores are killed in about 15 min	Microbiological media, solutions, linens, utensils, dressings, equipment, and other items that can withstand temperature and pressure
2. Pasteurization	Protein denaturation	Heat treatment for milk (72°C for about 15 sec) that kills all pathogens and most nonpathogens	Milk, cream, and certain alcoholic beverages (beer and wine)
3. Dry heat			
a. Direct flaming	Burning contaminants to ashes	Very effective method of sterilization	Inoculating loops
b. Incineration	Burning to ashes	Very effective method of sterilization	Paper cups, contaminated dressings, animal carcasses, bags, and wipes
c. Hot-air sterilization	Oxidation	Very effective method of sterilization but requires temperature of 170°C for about 2 hr	Empty glassware, instruments, needles, and glass syringes
Filtration	Separation of bacteria from suspending liquid	Removes microbes by passage of a liquid or gas through a screenlike material; most filters in use consist of cellulose acetate or nitrocellulose	Useful for sterilizing liquids (enzymes, vaccines) that are destroyed by heat

1. Moist heat

- a. Boiling: 100°C at sea level
- disinfection (cannot be relied upon to kill spores)
- Thermal death time at 100°C:
  Bacillus subtilis: 15-20 min.
  Clostridium botulinum: 360 min.
  Bacillus coagulans: 1140 min.

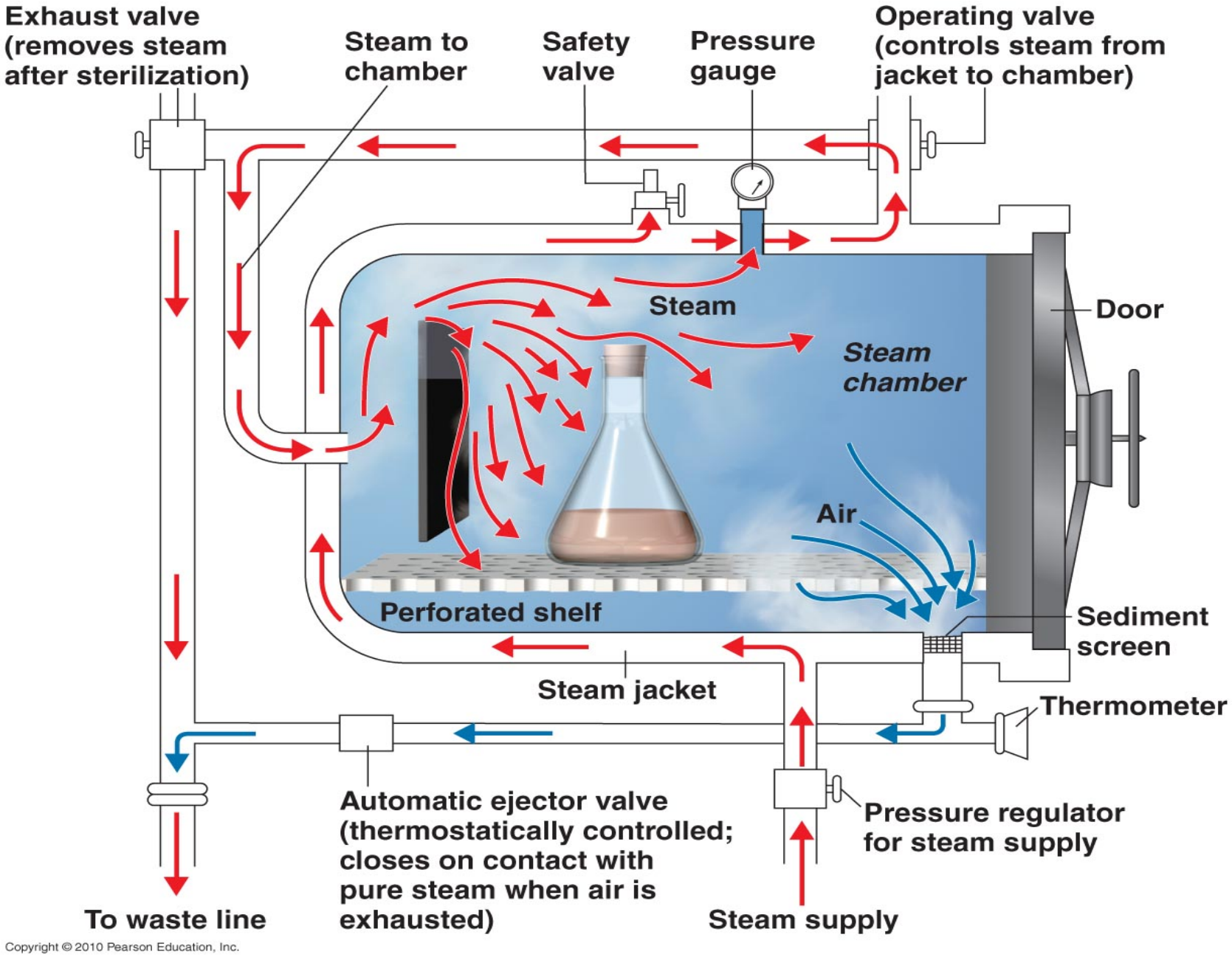
1. Moist heat

- a. Flowing steam: 100° C at sea level
- “instrument sterilizer”
- disinfection: does same as boiling

1. Moist heat

- b. Autoclaving - steam under pressure
  - function of pressure is to raise boiling point of water:
    0 psi = 100° C
    5 psi = 110° C
    15 psi = 121° C
    30 psi = 135° C
- steam under pressure used for sterilization: should kill spores, viruses, etc

2. Steam Autoclave



2. Pastuerization

- a). UHT treatment of milk (ultra high temperature): 140° C for 4 sec (STERILIZATION)
  - Fruit Juice, Cream, Soy Milk, Yogurt, Wine
  - Malliard browning

2. Pasteurization

- b. (HTST) 72° C for 15 seconds (75° if sugar in product); higher temps often used (Milk)
- should remove all pathogens
- allowing only organisms that can withstand heat to survive:
  - *Streptococcus lactis*
  - *Lactobacillus spp.*
  - some *Bacillus* species.

Louis Pasteur

3. Dry heat

a) Direct Flaming: sterilization

b) Incineration: sterilization

c) Hot air oven: sterilization
common run: 170° C (350° F) for about 2 hours

- dry heat requires longer times because for same temp, it carries less heat.

4. Filtration

– physical removal of organisms from liquids

– does not generally harm the organisms.

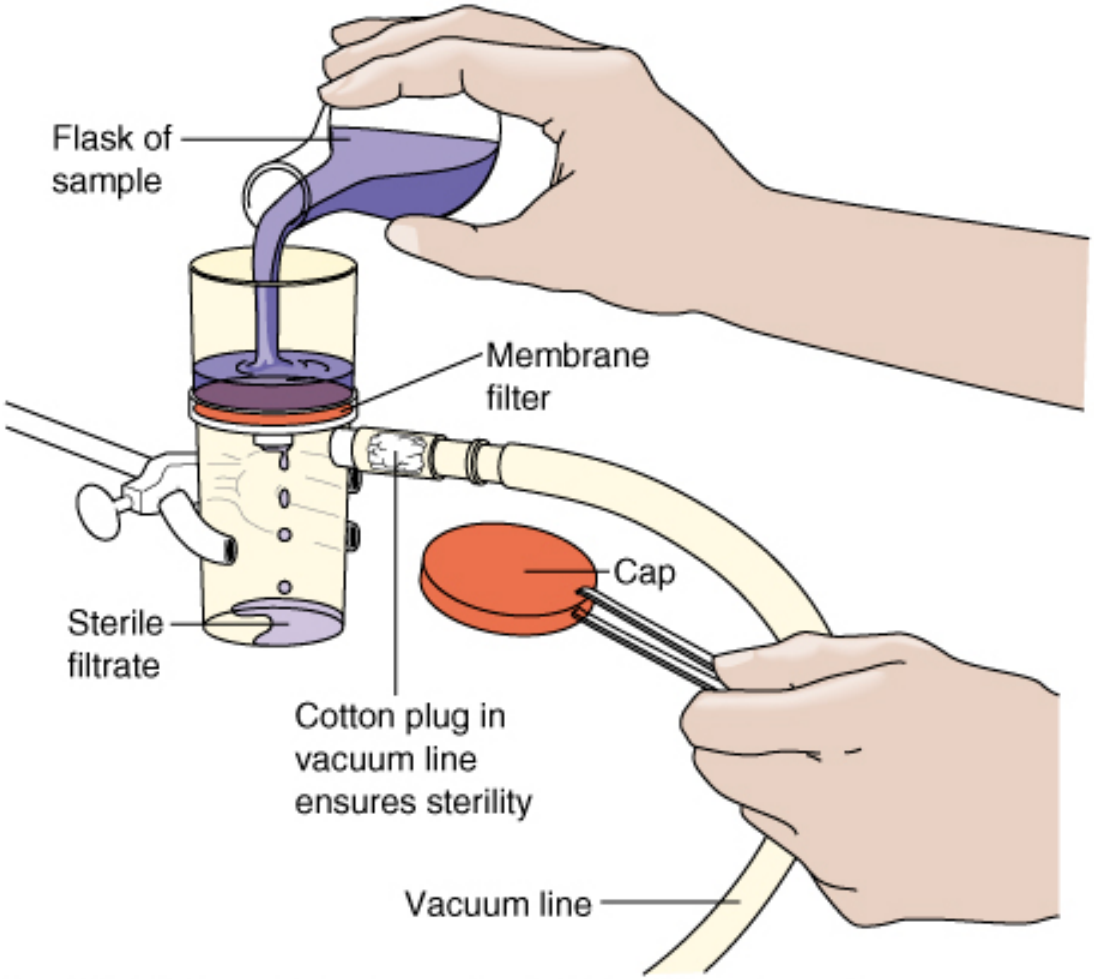
  - e.g. can filter beer as alternative to pasteurization; filtration is important in water treatment

– can get filters with specific pore sizes

– most filters pass viruses; a few remove them

– millipore filter:

Millipore membrane filter



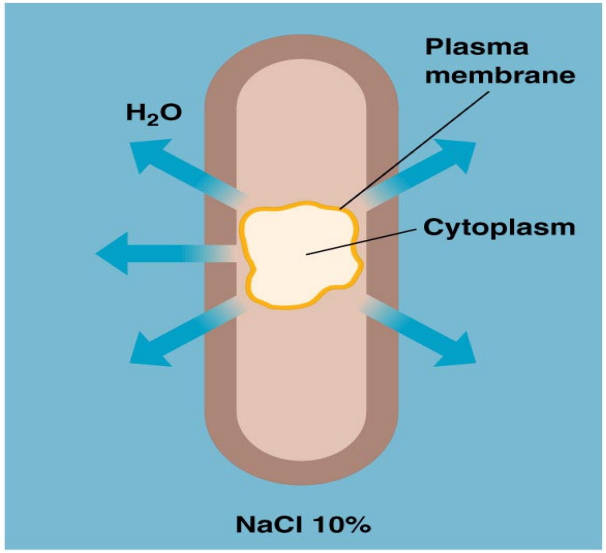
Filtration under pressure of vaccuum

Cold

- Static for most organisms
  - Listeriosis is an exception that can still grow in refrigerator
- refrigeration: optimum temp is just above freezing
- freezing: optimum temp for home freezer is 0° F (-17° C)

Drying (Desiccation)

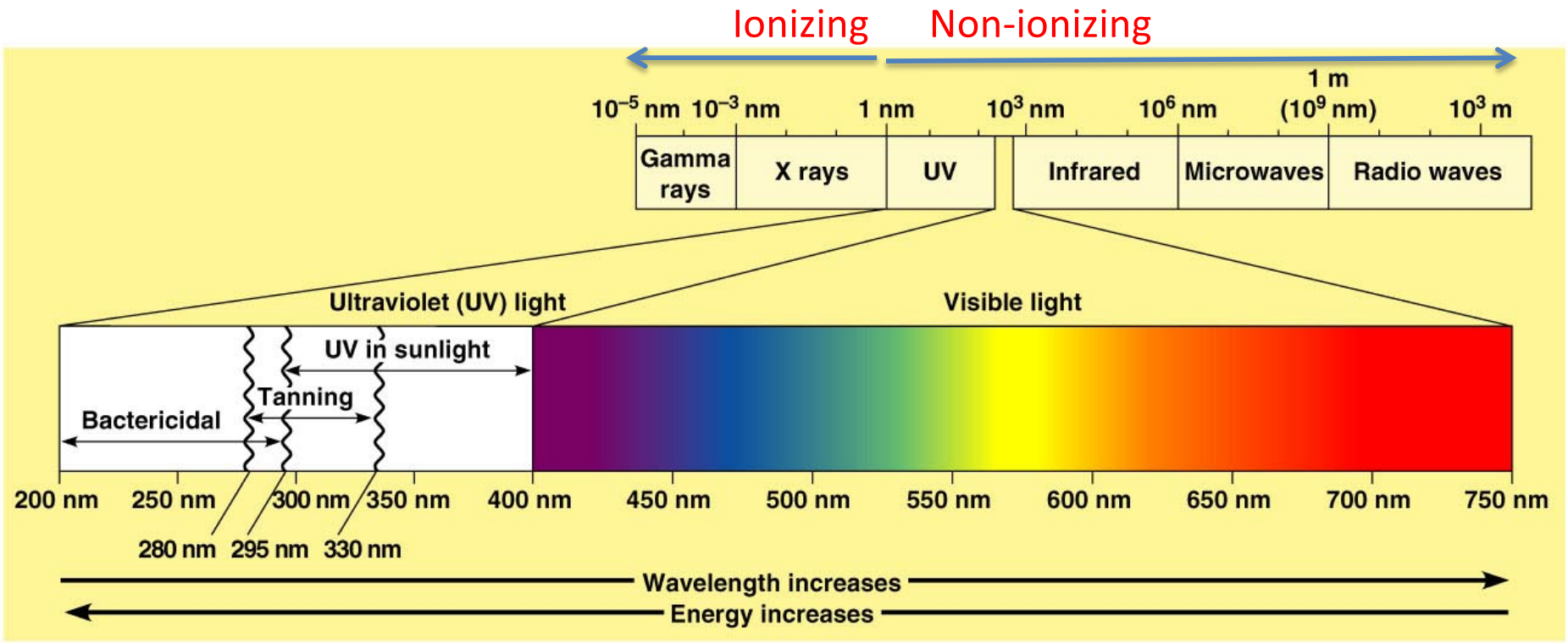
- static for most organisms
- e.g. drying fruits, salted fish and meats as method of preservation



(b) Plasmolyzed cell in hypertonic solution. If the concentration of solutes such as NaCl is higher in the surrounding medium than in the cell (the environment is hypertonic), water tends to leave the cell. Growth of the cell is inhibited.

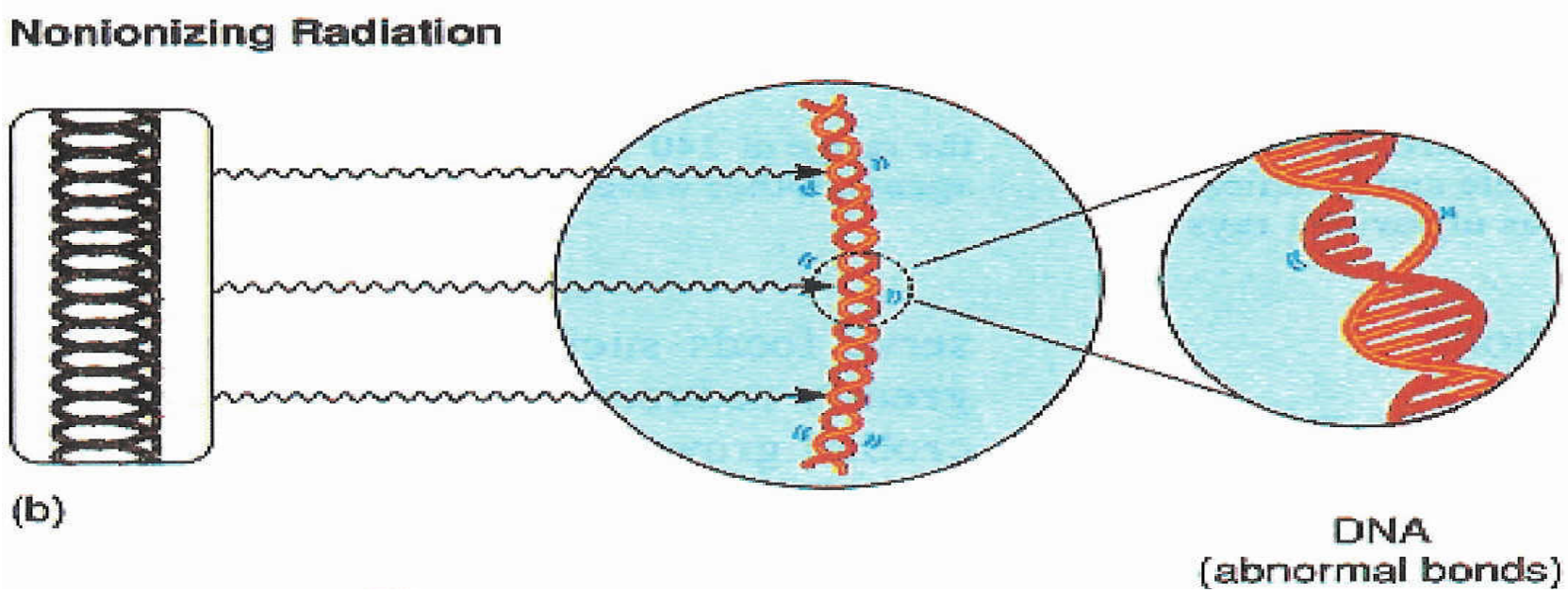
- lyophilization = freeze-vacuum drying
  - freeze item
  - dehydrate in powerful vacuum
  - static for most microorganisms
  - commonly used as a method of *preserving* microbial cultures.
  - Static

The Electromagnetic Spectrum
Radiation = Light



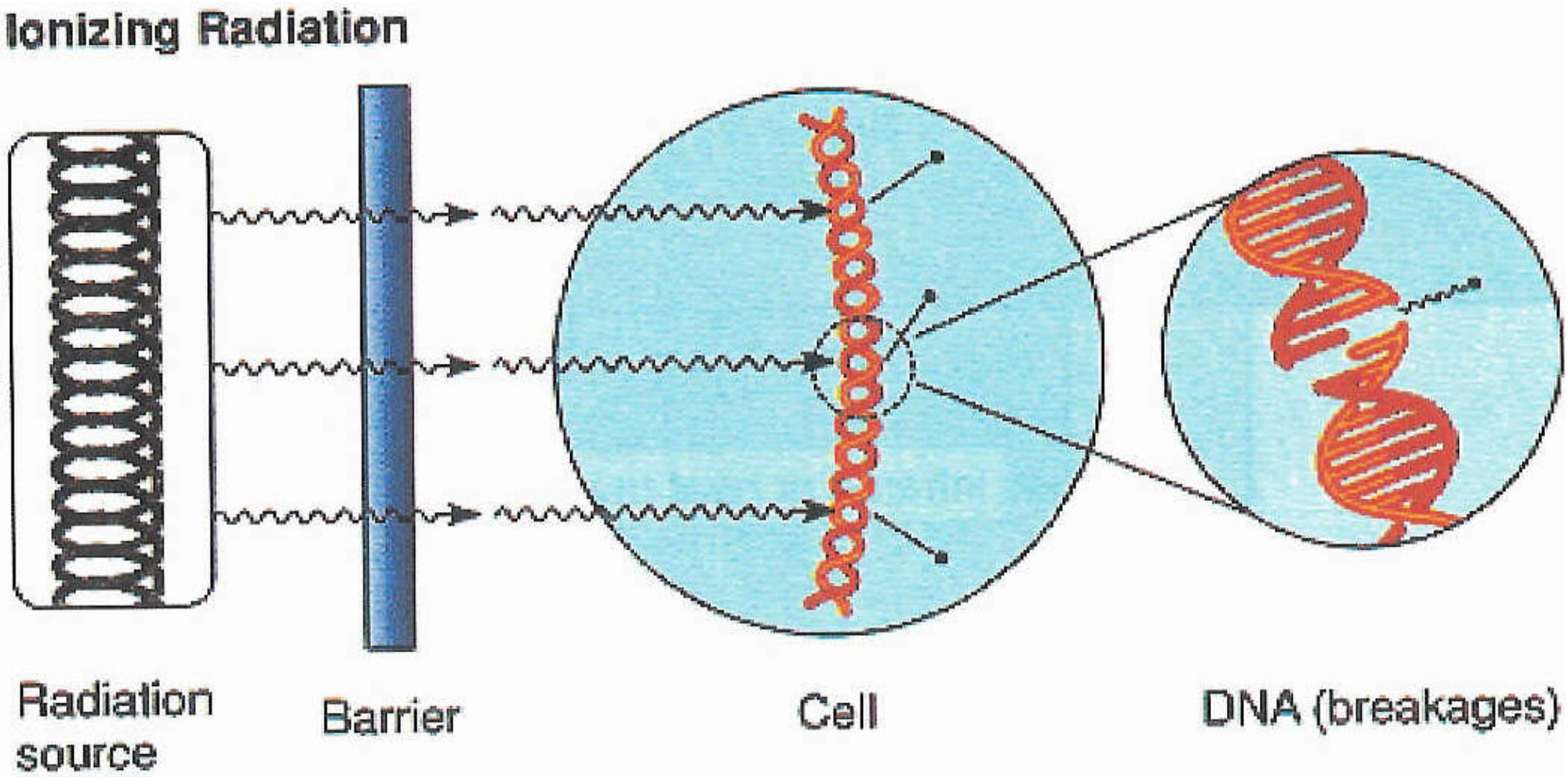
The electromagnetic spectrum: effective wave lengths:

- a. ultraviolet radiation



  - optimum wave length: 260 nm
  - Cidal, but poor penetrating ability

effect of ionizing radiation



Cidal = Can Result in Sterilzation, penetrates well but takes time…results in formation of free radicals

How about microwaves?

- Is yours a sterile environment ???
- Static for most bacteria
- Mositure containing foods Cidal for vegetative cells