General Microbiology
Bacterial sterilization and disinfection
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Disinfection

Destruction/Removal of Harmful Microorganisms

Disinfection is the elimination of pathogens, except spores, from inanimate objects.

- Disinfectants are ch[illegible]sed to clean inan[illegible]
  [illegible]s, e.g.. UV radiation, m[illegible] employed to effect disinfection)
- Germicides a[illegible]
  [illegible] and [illegible] for the purpose of [illegible] pathogens
- Antiseptics are formulated for application to living tissue

The Ideal Disinfectant

- Resistant to inactviation
- Broadly active (killing pathogens)
- Not poisonous (or otherwise harmful)
- Penetrating (to pathogens)
- Not damaging to non-living materials
- Stable
- Easy to work with
- Otherwise not unpleasant

Disinfectant Performance…

- Is dependent on Disinfectant concentrations
- Is dependent on length (time) of administration
- Is dependent on temperature during administration

  (usual chemical reaction 2x increase in rate with each 10°C increase in temperature)
- Microbe type (e.g., mycobacteria, spores, and certain viruses can be very resistant to disinfection—in general vegetative cells in log phase are easiest to kill)
- Substrate effects (e.g., high organic content interferes with disinfection—stainless steel bench easier to disinfect than turd)
- It is easier (and faster) to kill fewer microbes than many microbes

Cleansing

Cleansing is the removal of soil or organic material from instruments and equipment & may be done, clinically, in four steps:

- Rinsing the object under *cold* water
- Applying detergent and scrubbing object
- Rinsing the object under warm water
- Drying the object prior to sterilization or disinfection

Sterilization

Sterilization is the total elimination of all microorganisms including spores

- Typically the last things to die are the highly heat- and chemical-resistant bacterial endospores
- Instruments used for invasive procedures must be sterilized prior to use
- Moist heat or steam, radiation, chemicals (e.g., glutaraldehyde), and ethylene oxide (a gas) are employed for sterilization
- Sterilization by autoclaving, which uses moist heat, is used in most hospital and microbiology laboratory settings

Other Terms

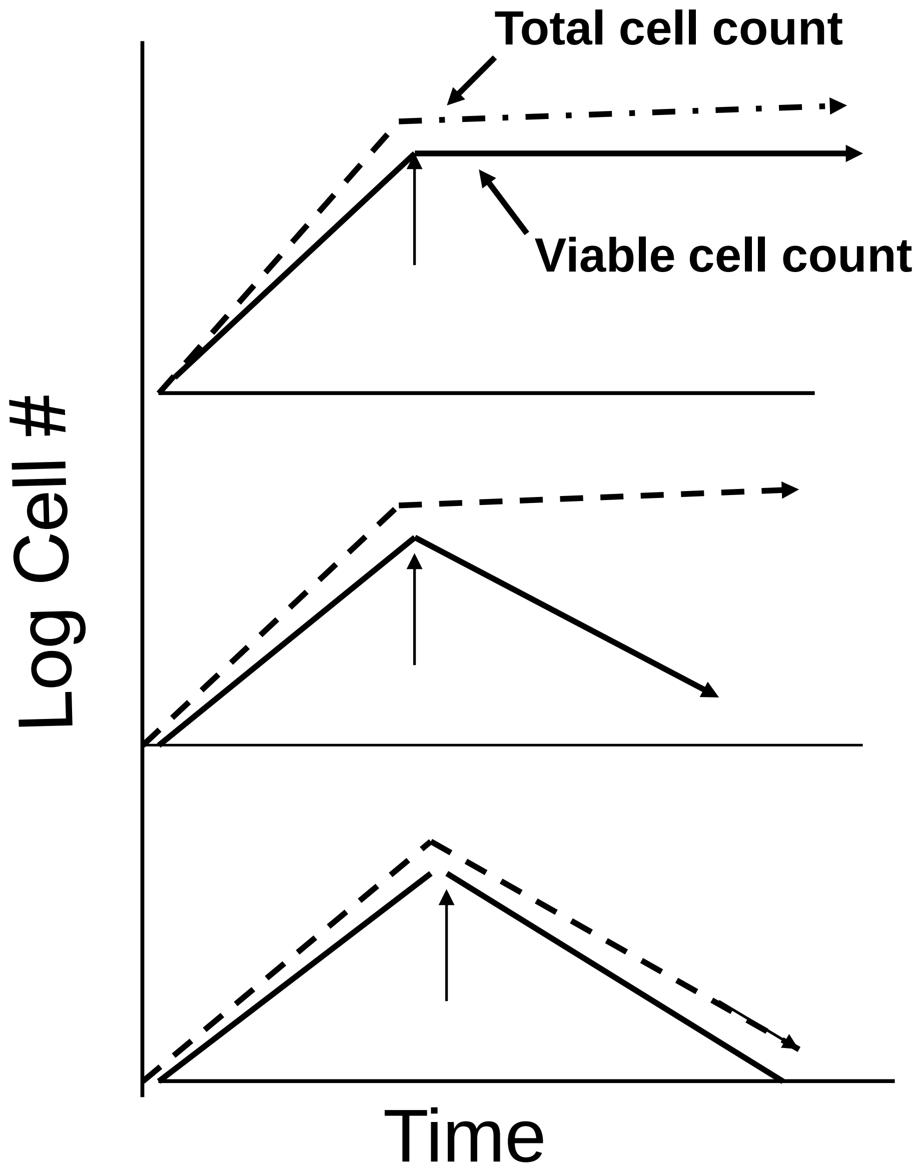
- Sanitization: Lowering of microbial counts to prevent transmission in public setting (e.g., restaurants & public rest rooms)
- Degerming: Mechanical removal of microbes, e.g., from hands with washing
- Sepsis: Bacterial contamination
- Antisepsis: Reduction of or Inhibition of microbes found on living tissue
- Germincides, Fungicides, Virucides
- Physical versus Chemical disinfectants
- Static (stasis) versus Cidal (e.g., bacteriostatic versus bacteriocidal)

Different Kinds of Bacteria "Death"

1. Bacteriostatic

2. Bacteriocidal

3. Bacteriolytic



Resistance to Killing

- Gram-negative bacteria (with their outer membrane) are generally more resistant than gram-positive bacteria to disinfectants and antiseptics
- Stationary-phase (I.e., non-growing) bacteria generally are more resistant than log-phase (I.e., growing) bacteria
- Mycobacteria, endospores, and protozoan cysts and oocysts are very resistant to disinfectants and antiseptics
- Nonenveloped viruses are generally more resistant than enveloped viruses to disinfectants and antiseptics
- Organic matter (such as vomit and feces) frequently affects the actions of chemical control agent
- Disinfectant activity is inhibited by cold temperatures
- Longer application times are preferable to shorter
- Higher concentrations, though, are not always preferable to lower concentration (e.g., alcohols)

Chemical Antimicrobials

Agent	Mechanisms of Action	Comments
Surfactants	Membrane Disruption; increased penetration	Soaps; detergents
Quats (cationic detergent)	Denature proteins; Disrupts lipids	Antiseptic - benzalconium chloride, Cepacol; Disinfectant
Organic acids and bases	High/low pH	Mold and Fungi inhibitors; e.g., benzoate of soda
Heavy Metals	Denature protein	Antiseptic & Disinfectant; Silver Nitrate
Halogens	Oxidizing agent Disrupts cell membrane	Antiseptic - Iodine (Betadine) Disinfectant - Chlorine (Chlorox)
Alcohols	Denatures proteins; Disrupts lipids	Antiseptic & Disinfectant Ethanol and isopropyl
Phenolics	Disrupts cell membrane	Disinfectant Irritating odor
Aldehydes	Denature proteins	Gluteraldehyde - disinfectant (Cidex); Formaldehyde - disinfectant
Ethylene Oxide	Denaturing proteins	Used in a closed chamber to sterilize
Oxidizing agents	Denature proteins	Hydrogen peroxide – antiseptic; Hydrogen peroxide – disinfectan; Benzoyl peroxide – antiseptic

Physical Antimicrobials

Agent	Mechanisms of Action	Comments
Moist Heat, boiling	Denatures proteins	Kills vegetative bacterial cells and viruses Endospores survive
Moist Heat, Autoclaving	Denatures proteins	121°C at 15 p.s.i. for 30 min kills everything
Moist Heat, Pasteurization	Denatures proteins	Kills pathogens in food products
Dry Heat, Flaming	Incineration of contaminants	Used for inoculating loop
Dry Heat, Hot air oven	Oxidation & Denatures proteins	170°C for 2 hours; Used for glassware & instrument sterilization
Filtration	Separation of bacteria from liquid (HEPA: from air)	Used for heat sensitive liquids
Cold, Lyophilization (also desiccation)	Desiccation and low temperature	Used for food & drug preservation; Does not necessarily kill so used for Long-term storage of bacterial cultures
Cold, Refrigeration	Decreased chemical reaction rate	Bacteriostatic
Osmotic Pressure, Addition of salt or sugar	Plasmolysis of contaminants	Used in food preservation (less effective against fungi)
Radiation, UV	DNA damage (thymine dimers)	Limited penetration
Radiation, X-rays	DNA damage	Used for sterilizing medical supplies
Strong vis. Light		Line-drying laundry

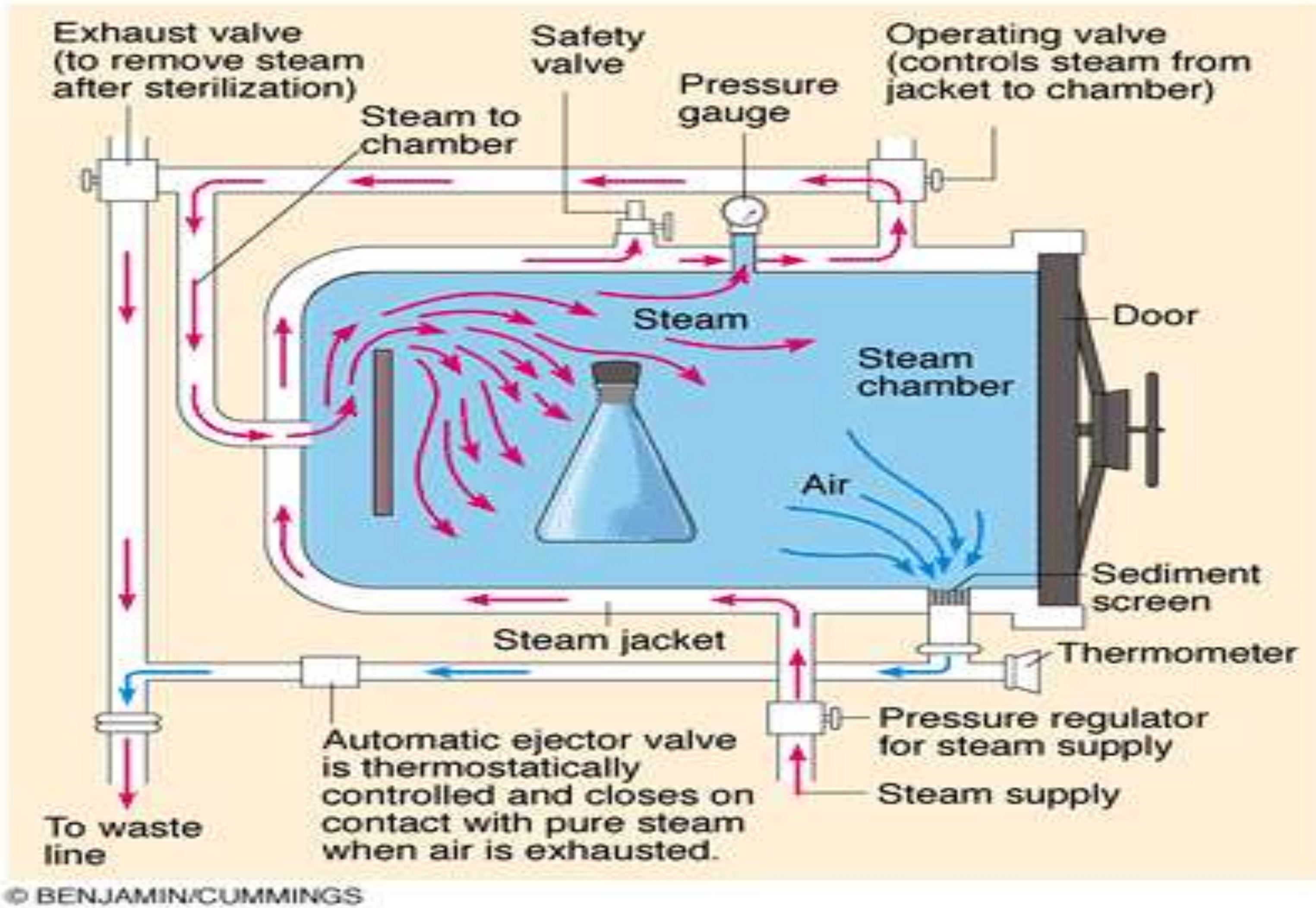
Application of Heat

- Heat is frequently used to kill microorganisms
- Thermal death point (TDP) is the lowest temperature at which all bacteria in a liquid culture will be killed in 10 minutes
- Thermal death time (TDT) is the length of time required to kill all bacteria in a liquid culture at a given temperature
- Decimal reduction time (DRT) is the length of time in which 90% of a bacterial population will be killed at a given temperature (especially useful in canning industry)
- Dry heat kills by oxidation (slow, uneven penetration)
- Moist heat kills by protein coagulation (denaturation) so requires lower temperatures or shorter times, but the moisture must penetrate to pathogens to be effective (grease & oil can block)

Moist Heat

- Moist heat kills microbes by denaturing enzymes (coagulation of proteins)
- Boiling (at 100°C, I.e., at sea level) kills many vegetative cells and viruses within 10 minutes
- Autoclaving: steam applied under pressure (121°C for 15 min) is the most effective method of moist heat sterilization—the steam must directly contact the material to be sterilized
- Pasteurization: destroys pathogens (*Mycobacterium tuberculosis*, *Salmonella typhi*, etc.) without altering the flavor of the food—does not sterilize (63°C for 30 seconds)
- Higher temperature short time pasteurization applies higher heat for a much shorter time (72°C for 15 seconds)
- An ultra-high-temperature, very short duration treatment (140°C for 3 sec.) is used to sterilize dairy products

Autoclave: Closed Chamber with High Temperature and Pressure



Filtration

Removal of microbes by passage of a liquid or gas through a screen like material with small pores. Used to sterilize heat sensitive materials like vaccines, enzymes, antibiotics, and some culture media.

- High Efficiency Particulate Air Filters (HEPA): Used in operating rooms and burn units to remove bacteria from air.
- Membrane Filters: Uniform pore size. Used in industry and research. Different sizes:
  - 0.22 and 0.45um Pores: Used to filter most bacteria. Don’t retain spirochetes, mycoplasmas and viruses.
  - 0.01 um Pores: Retain all viruses and some large proteins.

Filtration: Air & Fluids

Radiation

1. Ionizing Radiation: Gamma rays, X rays, electron beams, or higher energy rays. Have short wavelengths (less than 1 nanometer).

it causes mutations in DNA and produce peroxides.

Used to sterilize pharmaceuticals and disposable medical supplies.

Disadvantages: Penetrates human tissues. May cause genetic mutations in humans.

Radiation

2. Ultraviolet light: Wavelength is longer than 1 nanometer. Damages DNA by producing thymine dimers, which cause mutations.

Used to disinfect operating rooms, nurseries, cafeterias.

Disadvantages: Damages skin, eyes. Doesn't penetrate paper, glass, and cloth.

3. Microwave Radiation: Wavelength ranges from 1 millimeter to 1 meter.

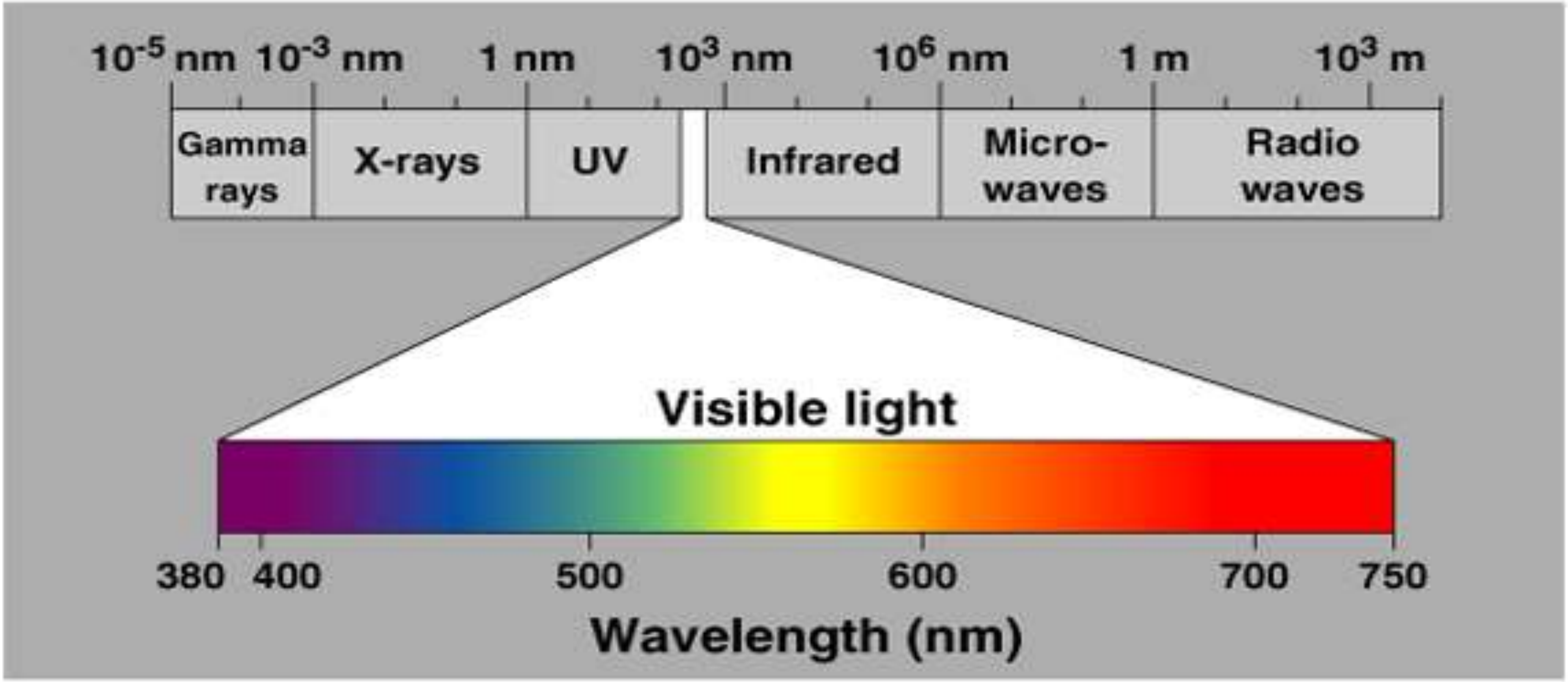
Heat is absorbed by water molecules.

May kill vegetative cells in moist foods.

Bacterial endospores, which do not contain water, are not damaged by microwave radiation.

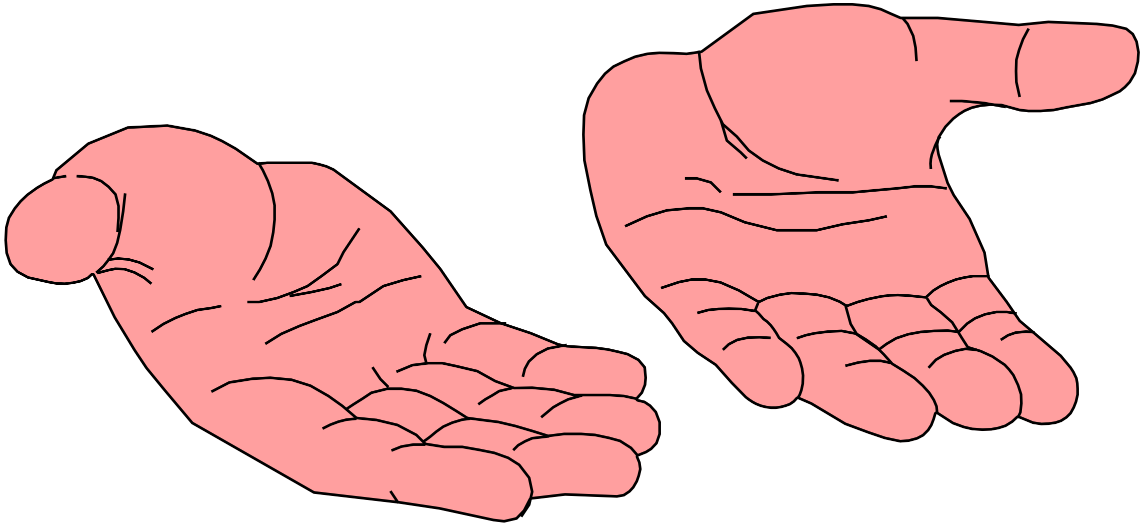
Solid foods are unevenly penetrated by microwaves.

Forms of radiation



Disk Diffusion Method

Hands Spread Disease



WASH 'EM!!!