Physical and Chemical Control of Microbes

Controlling Microorganisms

• The methods of microbial control used outside of the body are designed to result in four possible outcomes
  – sterilization
  – disinfection
  – decontamination (also called sanitization)
  – antisepsis

Sterilization
– The killing or removal of all microorganisms in a material or on an object including endospores.
  – Ex: autoclave or chemical sterilants

Disinfection
– Removes vegetative bacteria but not endospores
  – Ex: Bleach, iodine, boiling water

Decontamination (sanitization)
– Cleansing technique that mechanically removes microorganisms as well as other debris to reduce contamination to safe levels
  – Ex. Soaps, detergents

Antisepsis
– Reduces the number of microbes on the human skin. A form of decontamination but on living tissues.
  – Ex: Alcohol, surgical hand scrubs

Sepsis: the growth of microorganisms in the blood and other tissues

Asepsis:
– any practice that prevents the entry of infectious agents into sterile tissues and thus prevents infection
  – aseptic techniques: practiced in healthcare; range from sterile methods to antisepsis

Antiseptics: chemical agents applied directly to exposed body surfaces (skin and mucous membranes), wounds, and surgical incisions to prevent vegetative pathogens
  – preparing the skin before surgical incisions
  – swabbing an open sore
  – ordinary hand washing with a germicidal soap
More microbial control terminology

- Chemicals can be used to kill (-cide) or inhibit (-stat) microbial growth
  - Chemical agents are used on living tissues (as antiseptics) and on inanimate objects (as disinfectants).
  - Few chemicals achieve sterility.
  - Why can’t we always use disinfectants on our skin or mucous membranes?

3 Major Principles of Microbial Control

1. A definite proportion of the organisms die in a given time interval.
   - Not all microbes die immediately.
2. The fewer organisms present, the shorter the time needed to achieve sterility.
   - Think about cleaning up a mess. The bigger the mess, the more time it will take.
3. Microbes differ in their susceptibility to antimicrobial agents.
   - Need to match antimicrobial agents appropriately

Relative Resistance of Different Microbial Types to Microbial Control Agents

Effectiveness of Control Depends On:

- Number of microorganisms
- Target population (bacteria, fungi, spores, viruses)
- Temperature
- pH
- Concentration of agent
- Mode of action
- Interfering agents (solvents, debris, saliva, blood, feces)

Microbial Death

- Death: permanent termination of an organism’s vital processes
  - microbes have no conspicuous vital processes, therefore death is difficult to determine
  - permanent loss of reproductive capability, even under optimum growth conditions has become the accepted microbiological definition of death

How do we control microbial growth?

- Disinfection: The destruction or removal of vegetative pathogens but not bacterial endospores. Usually used only on inanimate objects.
- Sterilization: The complete removal or destruction of all viable microorganisms, used on inanimate objects.
- Antimicrobial: Compounds applied to body surfaces to destroy or inhibit vegetative pathogens.
Targets to Control Microbial Presence

- Injure cell wall
- Injure cell membranes
- Interfere with nucleic acid synthesis
- Interfere with protein synthesis
- Interfere with protein function
- Remove microbes

Which of the above would effect our cells too?

Cell Wall

- Bacteria and fungi
  - Block synthesis
  - Degrade cellular components
  - Destroy or reduce stability
- Agent
  - Chemical agent – Penicillin, detergents, alcohols
  - Physical agent –
    - Heat, radiation

Cell Membrane

- All microbes and enveloped viruses
  - Bind and penetrate lipids
  - Lose selective permeability (leakage)
- Agent
  - Chemical agent – Surfactants
  - Physical agent –
    - Heat, radiation

Nucleic Acid Synthesis

- Irreversibly bind to DNA
  - Stop transcription and translation
  - Cause mutations
- Agent
  - Chemical agent – formaldehyde
  - Physical agent –
    - Radiation, heat

Protein Synthesis

- Binds to ribosomes
  - Stops translation
  - Prevents peptide bonds
- Agent
  - Chemical agent –
    - chloramphenicol
  - Physical agent –
    - radiation, heat

Protein Function

- Block protein active sites
- Prevent binding to substrate
- Denature protein
- Agent
  - Chemical – alcohols, acids, phenolics, metallic ions
  - Physical – Heat
Physical Control Methods

- Temperature
  - Moist heat
  - Dry heat
  - Cold
- Radiation
  - Ionizing
  - Ultraviolet

Killing with Heat

- The most common method of sterilization.
- Modes of action:
  - Oxidizes proteins and nucleic acids
  - Denatures proteins/enzymes
- Effectiveness varies with: kinds of microbes, their number, intensity, length of exposure, pH, moisture, nature of product

Moist Heat Sterilization

- Most common and efficient method used
- Two kinds:
  - boiling
  - steam sterilization (autoclave)

Boiling

- Effective on glassware and instruments
- Kills fungi, protozoans, bacteria, viruses in 10-30 minutes
- Requires 3, separate boilings to kill endospores
- Can use at home
- Messy, time consuming, materials may require drying; endospores may require longer time
- Can you boil plastics items?

Steam Sterilization (Autoclaving)

- Uses: liquids, glassware, instruments, bandages, contaminated material
- Steam must reach all surfaces to be effective
- Most efficient and convenient. Kills all microbes in 15-20 minutes. Materials may require drying

Dry Heat

- Types:
  - Oven (hot air) sterilization
  - Flaming inoculating loops
  - Incineration/burning
- Temperature and time of exposure is greater than moist heat. Why would this be?
Dry Heat - Ovens
• Heat at 160-170 °C for 2 hrs.
• Kills microbes and endospores by oxidation or denaturation
• Used on glassware and instruments
• Can’t be used on liquid media, cloth, plastics, or articles wrapped in paper

Dry Heat - Incineration
• Destroys contaminated materials:
  – Blood soaked swabs, bandages
  – Contaminated objects and materials
  – Entire, diseased animal carcasses
• Safe and effective

Thermal Death Measurements
• Thermal death time (TDT): shortest length of time required to kill all test microbes at a specified temperature
• Thermal death point (TDP): the lowest temperature required to kill all microbes in a sample in 10 minutes

Radiation
• Movement of energy in waves through space and materials
• High frequency waves have the greatest penetrability
  – Waves strike molecules and knock out electrons
  – Releases ions and creates free radicals in cells
  – Ions attach to proteins and nucleic acids, damage cell structures, cause cell death
• Kills microbes on surfaces and within materials
• Good for heat-sensitive items.

Ultraviolet (UV) Radiation
• Moderate wavelengths, low penetrability. Won’t penetrate paper, glass or skin.
• Kills microbes on surfaces
• Cross-links DNA, inhibits replication, not safe to use on skin, causes burns, cancer
• Uses: sterilize surfaces (floors, walls etc) in labs and operating rooms. Also vaccines, serum, toxins, drinking water and waste water
  – Germicidal lamp in hospitals, schools, food preparation areas (inanimate objects, air, water)

A UV treatment system for disinfection of water
High Energy Ionizing Radiation

• Gamma rays, X-rays, Electron beams
• Gamma rays used to sterilize glassware, surgical instruments, sterile drapes
• Electron beams used to sterilize pharmaceuticals, disposable plastic syringes, surgical gloves, etc.

Mechanical Control Methods

• Filtration
  • Liquid
  • Gas

Filtration

• The passage of liquids and gases through screen-like material with pore sizes small enough to retain microbes.
• Removes microbes. Doesn’t kill or inhibit.
• Used to sterilize air and heat sensitive material.

Filtration and Filters

• Gases are forced through under positive pressure.
• Liquids are either forced through under pressure or pulled through under vacuum.
• Fluids are collected in sterile vessels

Uses of Filtration

• Heat sensitive material. i.e., plasma, sugar solutions, intravenous solutions, vaccines, antibiotic solutions
• Removes bacteria, but not viruses, from plasma. Slow because of plasma’s high viscosity

Air Filtration

• Used in operating rooms, burn units, laminar flow hoods in high security pathogen research. Also in rooms housing TB patients
• Use High Efficiency Particulate Air (HEPA)
Chemical Agents in Microbial Control

- Range from disinfectants and antiseptics to sterilants and preservatives

- **Aqueous solutions**: chemicals dissolved in pure water as the solvent

- **Tinctures**: chemicals dissolved in pure alcohol or water-alcohol mixtures

Chemical Agents in Microbial Control: Principles of Effective Disinfection

- Careful attention should be paid to the properties and concentration of the disinfectant to be used.

- The presence of organic matter, degree of contact with microorganisms, and temperature should also be considered.

Chemical Agents in Microbial Control: Selecting a Disinfectant

- Weigh the risks and benefits for each situation

- An ideal disinfectant should have:
  - Antimicrobial activity
  - Solubility
  - Stability
  - Lack of toxicity for humans and animals
  - Minimum activity by extraneous material
  - Activity at ordinary temperatures
  - Ability to penetrate
  - Material safety
  - Availability and low cost

Serial-Dilution Test

- The minimum inhibitory concentration (MIC) is the concentration required to inhibit growth of a specific isolate in vitro under standardized conditions.

- It is determined by finding the lowest dilution without visible growth during serial dilution testing. This will vary for individual isolates.

Disk-Diffusion Test

- Serial-dilution test
  - Calculates # of surviving microbes after 10 minutes in disinfectant solution

- Disk-diffusion test
  - Filter paper disk is soaked with disinfectant/antibiotic and applied to inoculated plate. Look for inhibited growth.
Chemical Agents in Microbial Control: Types of Chemical Control Agents

• You won’t need to know specifics about any of the chemical control agents. This is more for your information 😊

• Phenols
  - Dr. Lister used phenol to control surgical infections
  - Rarely used today because it is a skin irritant

• Phenolics
  - Often used because they are stable and persist for long periods
  - Example: Lysol

Types of Chemical Control Agents

• Halogens
  - Iodine
    - Betadine
    - Used for skin disinfection and wound treatment
    - Also used for water treatment
  - Chlorine
    - HOCl—hypochlorous acid → bleach (calcium hypochlorite)
    - Used to disinfect instruments and water
    - 10% bleach in water—good disinfectant, but needs to be fresh

• Alcohols
  - Disinfect and then evaporate
  - Used to degerm (remove microbes by swab)
  - Tinctures = solutions of disinfectant in alcohol
  - Alcohol based hand sanitizer rubs—effective alternative to hand washing

• Heavy Metals
  - Silver nitrate—used in many applications, for instance, in eye-drops applied to infants to protect against gonococcal infections which may cause blindness
  - Copper sulfate—destroys algae in ponds/pools

Demonstration of the Action of Heavy Metals

Types of Chemical Control Agents

• Quaternary Ammonium Compounds (Quats)
  - Most widely used surface-active agents (foamy)
  - Bind and disrupt cell membrane

  • Zephran
    - Disinfectant when concentrated, but can be used in dilute form as an antiseptic

  • Cepacol
    - Antiseptic and antiseptic
    - Mouthwash

Phenolics

• Vary based on functional groups attached to the aromatic ring

• Examples:
  - Hexachlorophene, Triclosan
    - Microbicidal
    - Ingredient in soaps to kitty litter
      - Disrupts cell walls and membranes,
Types of Chemical Control Agents

- Chemical Food Preservatives
  - sulfur dioxide—wine-making
  - sodium benzoate—prevent molds in acidic foods
  - sodium nitrate—meat product additive
  - Link between increased levels of nitrates and increased deaths from certain diseases including Alzheimer’s, diabetes mellitus, and Parkinson’s; possibly through the damaging effect of nitrosamines on DNA. (De La Monte, SM; Neusner, A; Chu, J; Lawton, M (2009). “Epidemilogical trends strongly suggest exposures as etiologic agents in the pathogenesis of sporadic Alzheimer’s disease, diabetes mellitus, and non-alcoholic steatohepatitis.” Journal of Alzheimer’s disease: JAD 17(3):519–29)

- Aldehydes
  - Very effective
  - Formaldehyde & glutaraldehyde—used to disinfect hospital instruments, however carcinogenic
  - Used by morticians for embalming

- Gaseous Chemosterilizers (good for heat-sensitive items)
  - Chemicals that sterilize in a closed chamber
  - Ethylene oxide—used on hospital equipment
  - Reacts with functional groups of DNA and proteins
  - Sterilizes and disinfects plastic materials

- Peroxogens
  - Hydrogen peroxide—better used on inanimate objects vs. open wounds
  - Benzoyl peroxide—treat acne by killing anaerobic bacteria in hair follicles
  - Peracetic acid—effective, considered a sterilant
  - Used in food processing and medical equipment

- Desiccation
  - At ambient temperatures
  - Essentially bacteriostatic
    - Kills many microbes (species sensitive)
    - Used to preserve foods, meats
    - Not a reliable sterilant!! Does not kill endospores and protozoan cysts.
    - In the lab it is used to store microbes

- Freeze Drying - Lyophilization
  - Slow freezing under vacuum removes water without ice crystal formation in cells.
  - Avoids cell damage.
  - Used to store bacteria and viruses as powders. Lyophilized microbes can be rehydrated and grown in culture.

Controlling Microbes in Food and the Lab: Refrigeration and Freezing

- Bacteriostatic: Inhibits growth and toxin production
- Slow freezing kills many microbes, but not all.
- Survivors multiply when returned to growth temperatures. Toxins produced previously are not affected.
- Not a Sterilant!!
- In the lab it is used to store microbes
Controlling Microbes in Food: Microwave Radiation

- Kills bacteria by heating.
- Unreliable sterilant!!
  - Ovens have “cold spots”
  - Materials must be rotated to achieve even temperature distribution.
  - Won’t kill *Trichinella* cysts.
- A new version for lab use sterilizes media in 10 min.

Controlling Microbes in Food: Osmotic Pressure

- Adding large amounts of salt or sugar to foods creates a hypertonic environment for bacteria, causing plasmolysis.
- Pickling, smoking, and drying foods have been used for centuries to preserve foods.
- Osmotic pressure is never a sterilizing technique.

Controlling Microbes in Food: Pasteurization

- Disinfection of beverages
- Exposes beverages to 71.6 °C for 15 seconds
  - Stops fermentation
- Prevents the transmission of milk-borne diseases
  - *Salmonella*, *Campylobacter*, *Listeria*, *Mycobacteria*
- Examples: Milk industry, wineries, breweries

Controlling Microbes in Food: Pasteurization

- Need to maintain taste and appearance
- Mild heat is used to kill pathogens and reduce microbe populations in liquid food and beverages.
- Standard method: Heat beverages to 60-66°C for 30 minutes. Cool rapidly and store in sterile containers in cold.
- Flash pasteurization: Heat milk to 71.7°C for 15 sec

Controlling Microbes in Food: Gamma Rays

- Used on poultry, pork, fresh fruits, white potatoes, spices.
- Kills bacteria in food
- Eliminates insects
- Prevents premature sprouting of seeds
- Extends shelf life of foods
- May discolor food and/or alter taste
- Animals fed irradiated feed loss weight
- No demonstrated risk from residual radiation