Chapter 11 – Physical and Chemical Agents for Microbial Control*

*Lecture notes are to be used as a study guide only and *do not* represent the comprehensive information you will need to know for the exams.

11.1 Controlling Microorganisms

Control of microorganisms in this chapter refers to the reduction in numbers and/or activity of the total microbial flora on our skin, on surfaces, in food, or anywhere in our environment where the presence of microbes could lead to infection. It is done to prevent transmission of disease (e.g. slow down epidemics), to prevent contamination of undesirable microbes (lab media must first be sterilized in order to inoculate with pure cultures), and to prevent deterioration or spoilage of materials by microorganisms (e.g. pasteurization to preserve milk).

General Considerations in Microbial Control

Three categories of controlling microbial growth include: chemical, physical, and mechanical methods (fig. 11.1).

One key concept to ask yourself when studying this material is: which method of controlling microorganisms would be best for the application in question? For example: pasteurization is fine for milk, but insufficient for sterilizing surgical instruments (for which autoclaving would be a better method).

Relative Resistance of Microbial Forms

Assess the level of resistance of the microbes that are contaminating an area and/or causing infection: **highest resistance, moderate resistance, least resistance**.

Terminology and Methods of Microbial Control

Definitions (Table 11.2):

- **Death** is defined as the irreversible loss of the ability to reproduce. **Viable** organisms, including bacteria spores, are capable of multiplying; dead ones are not.
- **Sterilization** means removing or killing ALL the organisms and viruses on an object or in a material.
- **Disinfection/Decontamination** is the process of reducing pathogenic microorganisms in or on a material to a level where they are no longer a hazard. A **disinfectant** is any substance used for this purpose. Most disinfectants are not safe for human use, so any nontoxic substance that can be safely used on animal tissues is called an **antiseptic**. Examples:
  - Disinfectant: 5% bleach solution, cleaning a floor.
  - Antiseptic: Triclosan soap, cleaning your hands.
- **Sanitize** means to reduce a microbial population to meet accepted public health standards. (Similar to disinfection.)

-cide is a suffix used to indicate an agent that kills microorganisms, while ***-static*** means a substance that prevents microorganisms from growing (e.g. *bacteriostatic* vs. *bactericidal*).

Bactericide destroys bacteria, with the exception of those in the spore stage.
Fungicide is a chemical that can kill fungal spores, hyphae and yeasts.
Virucide is a chemical known to inactivate viruses.
Sporicide is a chemical agent capable of killing bacterial spores.
Biocide is a substance that kills all living things, but especially microorganisms.
Germicide or microbicide kills pathogenic microorganisms. It can be used on living tissue or nonliving materials. This is a general term.

Sepsis refers to *growth of microorganism*s or the presence of microbial toxins *in blood* and other tissues. **Asepsis** refers to any practice that prevents the entry of infectious agents into sterile tissues to prevent infection. Aseptic technique is practiced in the health care professions and in the laboratory as cultures are inoculated. It is also called **antisepsis**, and chemicals applied to wounds, surgical incisions, or exposed body surfaces are called **antiseptics**. (see above)

What Is Microbial Death?

Environmental or physical factors that allow organisms to grow can also be used to prevent their growth. Chemical agents can also be used to control or prevent the growth of microbes. These two can work together. Fig. 11.2 illustrates factors that influence the action of antimicrobial agents:

1. The number of microorganisms. The higher the cell count, the longer it will take to destroy the microbial population.
2. The nature of the microbial population. Are resistant forms present? (e.g. spores: Table 11.4)
3. The temperature and pH of the environment.
4. The concentration of the antimicrobial.
5. The **mode of action** means how something works on a bacterium. Does it act on the nucleic acids, the cell membrane, the cell wall, etc.?
6. The presence of solvents, interfering organic matter or inhibitors.

How Antimicrobial Agents Work: Their Modes of Action

The **mode / mechanism of action** is the cell target of the chemical or physical method used. The cell targets are:

- **Cell wall** – disrupt the cell wall and/or inhibit cell wall synthesis of bacteria and fungi.
- **Cell membrane** – disrupt the chemistry of the phospholipids and proteins, can use **surfactants** and antibiotics.
- **Protein and nucleic acid synthesis** – disrupt the function of biochemical pathways, enzymes, and cell structures involved in producing nucleotides and amino acids.
- **Protein function** - modes that affect protein function that causes the protein to **denature** (fig. 11.5).

11.2 Physical Methods of Control: Heat

Effects of Temperature on Microbial Activities

All microbes have a temperature range over which they grow. Each has an optimum, minimum and maximum growth temperature. Temperatures above maximum generally kill. Temperatures below minimum inhibit metabolic growth and may even preserve the bacteria. High temperatures combined with high moisture are the most effective ways of killing bacteria. The mode of action is **protein denaturation**[1] (fig. 11.5), membrane disruption, and nucleic acid damage.

Thermal death time refers to the shortest period of time to kill a suspension of bacteria or spores at a prescribed temperature and under specific conditions (Tables 11.3/4). For example: it takes only 1 minute to kill *Bacillus subtilis* at 121°C in moist heat but 120 minutes at 121°C in dry heat.

The two procedures employed for high heat are **moist heat** and **dry heat**:

A. **Moist heat**

Moist heat works faster and at lower temperatures than dry heat. There are several variations of using moist heat including:

(i) **Autoclaving** – moist heat in the lab is "steam under pressure" or autoclaving (fig. 11.6). This is the most effective means to sterilize laboratory media and equipment. Autoclaves operate at 121° C @ 15 lb/in^2 of pressure for varying times (10-15 minutes for test tubes; up to 30 minutes for a liter flask of medium). Most liquid media used in the lab are autoclaved, but some are too sensitive to the high heat; other methods must be used. Autoclaving works very well for most small lab equipment (beakers, test tubes, pipettes, etc.). Tattoo artists also use autoclaves for sterilizing needles and inks.

(ii) **Intermittent sterilization** (**tyndallization**) is for those media that cannot be heated above 100°C without being damaged (media containing sera, egg or carbohydrates). This method involves heating the material for 30-60 minutes to 100°C on three successive days with incubation periods (23-24 hours) in between so any contaminating spores can germinate into vegetative cells (which are then kill during the next round of heating). It is the vegetative cells that are destroyed.

(iii) **Boiling water** (disinfection) - Objects ***cannot*** **be sterilized** by this procedure, but they can be disinfected. Boiling water (100°C) for 30 minutes will all kill non-spore-forming pathogens, including tubercle bacilli and staphylococci. This method is reliable for disinfecting drinking water contaminated with non-spore forming bacteria and protozoans. .

(iv) **Pasteurization** kills certain types of organisms products for human consumption, but ***does not sterilize*** the product. Pasteurization helps to control tuberculosis (*Mycobacterium tuberculosis*), listeriosis (*Listeria monocytogenes*), undulant fever (*Brucella*), Q fever (*Coxiella burnetii*), and

[1] **Protein denaturation** – when a protein is denatured, it is inactivated. Salt, acids, chemicals, heavy metals, and heat can all denature proteins. Heat denaturation causes proteins to unfold; you can witness this by frying an egg: as the egg cooks, the proteins which make up the egg white denature and change shape; you see this as the white changing from translucent to opaque white.

Campylobacter and *Salmonella* infections. Best known as a method for extending the storage time of milk, pasteurization can also be used on fruit juices, beer, wine, and even eggs. Pasteurization is conducted at 63-66°C for 30 minutes (batch method) or 71.6°C for 15 seconds (flash method). The second method is preferable for maintaining flavor and nutrient content. The bacterial count for vegetative stages of bacteria and fungi is reduced 97-99%, but is important to remember that pasteurization does NOT sterilize[2]. Even a refrigerated, never opened container of milk will eventually spoil.

B. **Dry heat**

(i) **Hot air sterilization** (ovens) is often used on glassware. A 2-4 hour exposure at 150-180°C (300-350°F) is sufficient for sterilization. The mode of action for dry heat is dehydration, denaturation of proteins and breakdown of DNA. However, since proteins are more stable in dry heat, it requires higher temperatures to inactivate them.

(ii) **Incineration** in a flame or electric heating coil is the procedure used in the lab on inoculation needles and loops. A Bunsen burner reaches 1870°C at its hottest point. Furnace incinerators operate at 800-6500°C. Incineration is often used as a method of disposal for medical waste.

The Effects of Cold and Desiccation

Cold temperatures (-20°C – 15°C) will slow growth, but it does not necessarily stop growth or kill microbes.

Desiccation is the removal of water from microbial cells which causes metabolic activity to stop, followed by a decline in the total viable population. The time of survival of microorganisms after desiccation varies. Examples: G- cocci (gonococci and meningococci) are very sensitive to desiccation, dying in a matter of hours. Streptococci are more resistant, some surviving for weeks. *Mycobacterium tuberculosis* in dried sputum remains viable for 6 weeks. Spores from many species can remain viable indefinitely.

Although desiccation can be used to preserve foods, numerous resistant microbes (micrococci, coliforms, staphylococci, salmonellae and fungi) survive in dried milk and eggs, which then spoil when rehydrated.

Lyophilization is the combination of freezing while drying (desiccating). This method is often used for long term storage of material. Lyophilization is generally not considered a sterilization or disinfection process.

[2] Even after pasteurization, milk might contain more than 20,000 microbes per milliliter!

11.3 Physical Methods of Control: Radiation and Filtration

Radiation as a Microbial Control Agent

Energy is transmitted through space in a variety of forms called **electromagnetic radiation**. Ultraviolet light (UV), x-rays, and gamma rays represent radiation at three different wavelengths , and each can be used to control microorganisms under different circumstances. Radiation kills microbes by causing varying degrees of damage to nucleic acids; DNA in particular (fig. 11.7).

Ionizing Radiation: Gamma Rays, X Rays, and Cathode Rays

Gamma rays

Gamma radiation is very high energy and is emitted by radioactive isotopes such as cobalt-60. Gamma rays are a type of **ionizing radiation** (fig. 11.8a). They penetrate deeper into matter and are lethal to all forms of life (biocide), including microbes. The gamma rays **cut DNA**; thus render it incapable of supporting the life of the cell. They have commercial value in sterilizing heat-sensitive packaged foods (the gamma rays will sterilize food, not cook it) and medical devices. Since, unlike UV radiation, the gamma radiation will easily pass through typical food packaging material. It is important to note that gamma rays do not make the food "radioactive", and are perfectly safe to use for this purpose.

X-rays

X-rays are lethal to microorganisms, but they are expensive to use, are difficult to utilize efficiently, and are dangerous to people. They are also used experimentally to produce microbial mutants.

Nonionizing Radiation: Ultraviolet Rays

Ultraviolet Light (Non-ionizing radiation)

UV light ranges from 150 to 3900 Å (fig. 11.7 and fig. 11.8). Wavelengths of 2650 Å (265 nm) have the highest bactericidal efficiency. Lamps that emit a high concentration of UV light at 2600 to 2700 Å are called **germicidal lamps**. Ultraviolet light is absorbed by DNA where thymine nitrogenous bases are cross-linked, thus creating **thymine dimers** (fig 11.10). When the DNA tries to replicate, the thymine dimers cause mutations[3] which result in loss of gene function and the eventual death of the microbe. UV lamps are used to reduce microbial populations in hospital operating rooms, in the food and dairy industries for the treatment of contaminated surfaces, in laboratories, and in water and wastewater treatment (fig 11.11). One drawback is that UV radiation can be easily blocked by solid barriers such as plastic (think sunglasses); as a result, UV radiation isn't effective for reducing microbe counts within packaged foods.

[3] UV light absorption by DNA in human skin cells can also lead to mutations and eventually skin cancer.

Filtration – A Physical Removal Process

Filtration is generally used on liquids that are heat-sensitive (fig 11.12). They allow the fluid to pass through but prevent the passage of bacteria. The pore diameters of the filter paper range from 8μm to 0.02 μm. Since filters can be used to trap bacteria, they are also a way of concentrating bacteria for isolation. For example, if there is one bacterium in a liter of water, it could be trapped on a sterile filter, the filter placed on a medium and incubated to produce a colony.

Unless the pore diameter is 0.02 μm or smaller, a filer is an ineffective means for removing viruses from solution since viruses are so small. For example, one virus which causes the common cold, adenovirus, is only 75 nanometers across; that is 0.075 μm – so if the filter pore is 0.1 μm, the virus will get through. Small pore filters are very expensive.

***Note*: Osmotic Pressure**
If microorganisms are exposed to a high solute concentration (hypertonic solution), water is drawn out of the cells in a process called plasmolysis. Salt is used in brines, pickled foods, meats and fish. It does not prevent the growth of organisms that prefer high salt environments (e.g. *Staphylococcus aureus* and *Listeria monocytogenes*) and typically only slows the growth of others. High sugar used in candies, jellies and canned fruits also preserves the food by osmotic pressure. High osmolarity is generally bacteriostatic. Bacteria are resistant to hypotonic environments due to their cell walls.

11.4 Chemical Agents in Microbial Control

See Tables 11.6, 11.7, 11.8, 11.9, 11.10, 11.11, 11.12 for details on chemical agents.

Chemicals can function as disinfectants, antiseptics, and sterilants.

Choosing a Microbicidal Chemical

Concerns for choosing a chemical is: work fast in low concentration, broad-spectrum, noncorrosive, affordable, and nonoffensive odor.

Factors That Affect the Germicidal Activities of Chemical Agents

LENGTH OF EXPOSURE Consideration must be taken when using the chemical on different surfaces and various combinations of body fluid presence.

CONCENTRATION OF THE CHEMICAL AGENT Every chemical has an optimum concentration for its effectiveness.

Categories of Chemical Agents

Halogens

The halogens are fluorine, bromine, chlorine, and iodine – nonmetallic elements that are commonly found in minerals, seawater, and salts.

(i) **Iodine** is one of the oldest and most effective germicidal agents and has been used for over a century. It is usually in a form referred to as **tincture of iodine** – 2% solution of iodine and sodium iodine in 70% alcohol. Iodine is effective against all kinds of bacteria, as well as being fungicidal and to some extent virucidal. It is used as a disinfectant on the skin. Iodine diffuses through the membrane of cells where denatures proteins by interfering with hydrogen and disulfide bonds. It is used as a topical antiseptic before surgery and occasionally as a treatment for burned and infected skin, although some people express some sensitivity to it.
Note: iodine solutions are lethal if taken internally, but low concentration iodine tablets are used to disinfect water of the protozoan *Giardia lamblia*.

(ii) **Chlorine**, as gaseous chlorine, as hypochlorite, as chloramines or in combination with other chemicals, is widely used as a disinfectant. Chlorines are used to control microorganisms in water treatment, the food industry, domestically, (e.g. Tilex™ , Chlorox™), and in medicine. Chlorine compounds have been used to disinfect open wounds, to treat athlete's foot (a fungal infection), and other infections, and as a general antiseptic. When chlorine comes in contact with water, hypochlorous acid is formed. It interferes with the sulfhydryl group on the amino acid cysteine and with disulfide bridges on numerous enzymes, thus denaturing them. Effective on bacteria, endospores, fungi and viruses. *Drawbacks*: ineffective if used at an alkaline pH, excess organic matter can reduce their activity, are relatively unstable, especially if exposed to light. Chlorine in swimming pools is an effective measure to prevent many contagious microbes, but it is important to note that some (e.g. the protozoan *Cryptosporidium*) can survive in properly chlorinated water for as long as 6 days.

(iii) Fluorine
Fluorine, like chlorine in its ionic state, is highly toxic to humans. However, compounds containing **sodium fluoride** are useful microbicidal agents and are commonly found in mouth rinses and toothpastes.

Phenols

Phenol (carbolic acid) is an acrid, poisonous compound derived from the distillation of coal tar. Joseph Lister first used it in the 1867 to reduce infection in surgical incisions and surgical wounds. Chemical derivatives of phenol – phenolics – may be either bactericidal *or* bacteriostatic, depending upon the concentration used. Bacterial spores and viruses are more resistant than are vegetative cells. Phenolics are aromatic carbon rings with added functional groups (fig. 11.13). Some phenolics are fungicidal. Chemical variations of phenol compounds are common in household products such as: **Triclosan** (dichlorophenoxyphenol) is used in antibacterial soaps (e.g. Softsoap™) and Orthophenyl phenol is a common ingredient in aerosol disinfectant sprays and in Lysol™. The mode of action for phenol is not well understood, but in sufficient concentrations physical damage to the cell membrane and cell wall occurs. In low concentrations, they inactivate enzyme systems.

Another variation of phenol using chlorine (see halogens below) – **chlorhexidine** – is now commonly used for such wide ranging applications such as: hand scrubbing, preparing skin sites for surgery or injections, a neonatal wash, wound cleanser, and as a preservative for eye solutions.

Alcohols

Ethyl and **isopropyl alcohols**, in concentrations of 50-95%, are effective against vegetative cells (Table 11.9). Concentrations above 50% dissolve membrane lipids, disrupt cell surface tension, and compromise membrane integrity. Alcohols that enter the cytoplasm denature proteins. 100% alcohol dehydrates cells and inhibits growth, but does not coagulate protein. A **70% ethanol**[4] in water solution **works best** since the water helps the alcohol pass through the membrane and therefore denature the bacterial proteins. Alcohol is effective in reducing the microbial flora of skin (is used in hand-sanitizer gels), for disinfecting oral thermometers, and in conjunction with iodine to prepare the skin for surgery or injections.

Hydrogen peroxide is a colorless, caustic liquid that decomposes in the presence of light, metals or catalase enzyme:

$$2H_2O_2 \rightarrow 2H_2O + O_2\uparrow$$

The germicidal effects of H_2O_2 are due to the direct and indirect actions of oxygen (Table 11.9). When H_2O_2 breaks down naturally, OH^- (called a hydroxyl free radical) is formed; this is highly reactive and destroys proteins. While catalase can neutralize small amounts of hydrogen peroxide inside a bacterium, it cannot neutralize the large amounts that enter the cell during disinfection and antisepsis. H_2O_2 is a versatile bactericide, virucide, and fungicide. Serves a variety of needs including skin and wound cleansing, bedsore care, and mouth washing. (Note: if you have ever cleaned a wound with hydrogen peroxide and notices bubbling, the bubbles where O_2 being released as the catalase in your cells broke down the H_2O_2 .

Aldehydes (including formaldehyde and glutaraldehyde, (Table 11.10) are bactericidal and sporicidal. These chemicals denature proteins and nucleic acids. Useful for the sterilization of certain instruments and can be used for the disinfection and sterilization of enclosed areas. It kills spores in 3 hours and fungi and vegetative cells in a few minutes. Viruses appear to be inactivated after relatively short time exposures. Also used as a preservative for dead tissues (anatomy specimens for example).

Gaseous agents, such as **ethylene oxide**, are used to sterilize medical devices that cannot be autoclaved (e.g. plastic syringes, pipettes, Petri dishes, etc.). They are used in a Chemiclave (fig 11.15). Ethylene oxide reacts with guanine in DNA and functional groups of proteins. *Drawbacks*: explosive, damages lungs, eyes and mucous membranes; rated as carcinogenic. A **chemiclave** is a variation on the autoclave. It uses ethylene oxide for the chemical sterilization process.

---

[4] 70 ml of alcohol + 30 ml H_20 = 100 ml of a 70% alcohol solution. This is what we use to disinfect the lab benches.

Detergents are polar molecules that act as surfactants (act on surfaces) (fig 11.16; Table 11.11). Detergents insert into phospholipid bilayers and disrupt the cell membrane. Detergents are found in laundry and dishwashing soaps, shampoos, hand soaps, etc. Detergents are chemically classified as anionic, cationic and nonionic. Positively charged quaternary ammonium detergents are the most germicidal. **Soaps** are alkaline compounds made by combining fatty acids in oils with sodium or potassium salts. Alone, soaps are good degreasers and effectively remove dirt, but are only weakly microbicidal.

NOTE: Hand scrubbing (with germicidal soap) is still the best method for controlling the spread of infectious agents.

Heavy metals
Heavy metal such as mercury, silver, copper, cadmium, lead, and nickel combine with cellular proteins and inactivate them (fig. 11.17; Table 11.12). High concentrations of salts in heavy metals coagulate cytoplasmic proteins. They are *ineffect* rcurochrome (which is now considered a poor antiseptic). **Silver nitrate ($AgNO_3$)** once used to treat *ive* on endospores. Examples: mercury as mercuric chloride, merthiolate, me the eyes of newborns for neonatal gonococcal ophthalmitis has now been replaced by antibiotics, but is still used as a cauterizing and disinfecting agent on wounds. *Drawbacks*: metals, even in small quantities, are *toxic to humans* if ingested, inhaled or absorbed through the skin; cause allergic reactions; biological fluids and wastes neutralize their activity; microbes may develop resistance to the metals.

Dyes can not only be used in staining and selective / differential media techniques, they are also used interfere with microbial cell growth. Dyes can be used in ointments, for antisepsis and wound treatment. But, dyes have a narrow spectrum.

Acids and Alkalis Changing the pH of the microbe's environment can slow growth, and may stop growth. Sodium hydroxide is a chemical that can be very effective, even at low concentrations. Acetic acid, like vinegar, can inhibit microbial growth. See Table 11.13.