Control of Microorganisms

Microorganisms are abundant in the environment, living in soil, in water, on the surfaces of plants and animals and inside many types of multicellular organisms, including humans. Fortunately, most microorganisms live freely in the environment without causing harm to humans or other organisms; in fact most of them are beneficial in a variety of ways. For these reasons, humans seek to control only certain types of microorganisms and under relatively few specific circumstances. We exert control over microorganisms associated with **food materials**, so that foods can be stored and made available for human consumption without being consumed by microorganisms first. We seek to control microorganisms that might cause damage to **agricultural crops**, **forest trees** and **ornamental plants** maintained for our purposes, and we seek to control potential pathogens that threaten **our health or the health of animals** we consider important for various reasons. Microbial control methods vary considerably depending on where and on what types of microorganisms they are being applied. Some of the more commonly used control methods will be presented below.

Control methods can be divided into categories based on their effects, and the types of factors involved. Control methods or treatments that **kill cells** are given the prefix **cidal;** for example, **bactericidal** treatments/methods **kill bacteria**, while **fungicidal** treatments/methods **kill fungi**. Most people are familiar with the prefix cidal as it is applied to insecticides and herbicides used frequently (and often without consideration for potential consequences) throughout this country. Control methods or treatments that **inhibit microbial growth** but do not kill cells are given the prefix **static**. Static treatments, e.g., freezing, are effective only as long as they are maintained, but within the body, the inhibition of microbial growth for even a short time period often improves the effectiveness of immune mechanisms ultimately resulting in elimination of potential pathogens.

Sterilization – When used with reference to microbial control, the term sterilization refers to any treatment or method rendering an object or material virtually free of any viable cells. Skin surfaces cannot be sterilized because the skin is composed of living cells (even though the surface epithelial cells are mostly dead). Liquids, air, metal instruments and other materials can be sterilized by a variety of methods as described below.

Physical Factors Effective in the Control of Microorganisms:

A variety of microbial control methods involve physical factors such as temperature, pressure and radiation. Variations exist within these categories, and in some cases, more than one factor can be applied simultaneously. Some specific examples are listed below.

1. **Temperature**
    a. **Freezing** (exposure to low temperatures) **–** Freezing is a bacteristatic physical control method applied frequently in a wide variety of settings. Freezing is used extensively to control microorganisms associated with food materials, drugs, research chemicals, etc. Freezing effectively inhibits the growth of most microorganisms by stopping metabolic processes, but is rarely cidal to bacteria

because most of them are **psychroduric**. Bacteria culture collections are typically maintained in ultra low freezers (at temperatures of minus 72 to minus 80° C) and remain fully viable. It is important to remember when thawing large frozen food items (e.g., turkeys) that the external surfaces can reach temperatures suitable for microbial growth long before the center is thawed. Microorganisms and nutrients are typically abundant on skin surfaces and growth will occur if moderate temperatures are maintained for any length of time.

Cold temperatures commonly maintained within refrigeration units slow metabolic processes and effectively prevent the growth of some types of microorganisms, but not all. Potential pathogens including *Campylobacter* and *Listeria* can grow on food materials maintained at temperatures common within most refrigerators.

b. **Pasteurization** – Pasteurization is a control method developed by Lewis Pasteur and first tested in 1862. It involves the application of heat (at a specified temperature) for a specific period of time, and is intended to bring about a logarithmic reduction in the number of viable cells present, so that spoilage due to microorganisms is prevented, or at least slowed. Heat will denature enzymes essential to metabolism if applied appropriately. In our laboratory we Pasteurized soil samples by placing them into boiling water (100° C) for one minute. If lower temperatures are used, the exposure time is increased, and if higher temperatures are used the exposure time is decreased. Pasteurization was used initially to control microorganisms associated with fruit juices, but can also be used to control potential pathogens in milk, beer, vinegar and other food products.

   Although Pasteurization kills the vegetative cells of many pathogenic bacteria (*Mycobacterium tuberculosis*, *Escherichia coli*, *Salmonella cholerasuis*, and others), it cannot be used to sterilize liquids. Pasteurization does not kill **thermoduric endospores** or **hyperthermophilic** bacteria. Subjecting materials to boiling water for more than one minute (e.g., five minutes, ten minutes, etc.) is sometimes considered sufficient for sterilization, though technically it is not, because hyperthermophiles are not killed. Fortunately hyperthermophiles are rarely if ever pathogenic, so controlling them is not an issue.

c. **Tyndallization** – Tyndallization, also known as **fractional sterilization**, was developed by John Tyndall (1875-76), and was used to sterilize liquids before the development of autoclaves. Tyndallization involves subjecting liquids to alternate periods of boiling and cooling extended over three days. Liquids cannot be sterilized by simple boiling, even when it is applied for 30 minutes, but boiling for 30 minutes followed by cooling to room temperature overnight and then repeating this procedure for the next two days will result in sterilization. Maintaining liquids at room temperature typically causes endospores to germinate, and the resulting vegetative cells can then be killed during the next phase of boiling.

d. **Autoclaving** (moist heat) – An autoclave is a device (essentially a sophisticated pressure cooker) used to sterilize liquids, glass containers, pipette tips and other materials by subjecting them to steam heat under pressure. Autoclaves allow for the application to two physical factors (heat and pressure) simultaneously, and will effectively kill all types of cellular pathogens within minutes. Although modern autoclaves can typically be set to run different cycles, sterilization is usually

accomplished by raising the temperature to 121° C with steam under 15-17 pounds per square inch (psi) pressure, for 15-20 minutes. The endospores of *Bacillus stearothermophilus* can survive 121° C for about 13 minutes, but not longer, and are sometimes used to test the effectiveness of autoclaves.

Although autoclaves effectively kill pathogens, they involve considerable moisture (steam) that is potentially damaging to some metal instruments. Materials sensitive to moisture can be sterilized by subjecting them to dry heat.

e. **Dry heat** – Dry heat, as would be applied within an oven, is sometimes used to sterilize glassware or metal instruments potentially damaged by exposure to steam (although dry heat will also cause surgical instruments to loose sharpness). Dry heat applied at 160° C will sterilize materials in about 2 hours.

f. **Incineration** – Incineration, i.e., burning or exposing to open flame, can be used to sterilize materials such as wire loops and glass spreader rods, and is used routinely in the microbiology laboratory. Organic materials are vaporized during incineration and no living cells can withstand this treatment. Incineration was once commonly used as a method for disposing of corpses associated with large epidemics, and is still used for eliminating body parts, animals dead due to anthrax and microorganisms associated with large structures such as the filters used inside laminar flow hoods.

2. **Pressure**

As described above, autoclaves use heat and pressure simultaneously to kill microorganisms and sterilize various types of materials. Pressure is also a major factor used to kill and break open Gram-positive bacteria in preparation for the PCR activities carried out in our laboratory. When subjected to sufficient pressure, e.g., being slammed repeatedly by 2mm glass beads, bacterial cell walls will break. According to some sources, nanotechnology is being investigated as a means of applying pressure to the control of microorganisms on commonly used surfaces such as computer keyboards, instrument control panels, light switches etc. within clinical settings. If such surfaces could be equipped with nano-spines, the pressure exerted by human hands would result in cell death as the bacteria on skin surfaces were punctured. Exactly how the skewered bacteria would be removed from such surfaces is difficult to imagine.

3. **Radiation**

a. **Ultra violet light –** As described earlier, ultra violet light (electromagnetic radiation with a wavelength shorter than visible light), can cause the formation of thymine-thymine dimers resulting in deletion type point mutations, but can also cause chromosome distortion and inhibition of replication. Ultra violet light with a wavelength of 260-270nm is particularly effective in controlling bacteria and is often used in research laboratories, clinical settings and to reduce the number of viable bacteria in water exiting wastewater treatment facilities.

b. **Ionizing radiation** – Ionizing radiation such as X-rays and gamma-rays can be used to sterilize heat-sensitive materials such as plastics, vaccines, drugs, important

spices and other materials that would be damaged or destroyed by exposure to heat. Ionizing radiation is very effective, but used less commonly than other control methods because it is expensive and because it is potentially harmful to persons applying it. Treating food materials with ionizing radiation does not render them radioactive.

4. **Filtration**

   Filtration is a static control method used to sterilize liquids and gases. The organisms removed during filtration may eventually be killed by exposure to heat or some other treatment, but the filtration itself merely inhibits their growth by removing them from the potential growth medium. In our laboratory we filter sterilize certain media such as urea agar, arabinose, and xylose, as well as chemicals to be added to autoclaved media (e.g., ampicillin and $CaCl_2$). The air flowing through the laminar flow hood is filter sterilized to provide a clean space for the preparation of plate media. Beer is typically filtered to remove yeast cells prior to bottling.

Chemical Factors Effective in the Control of Microorganisms:

Many different chemicals are used to control microorganisms in a variety of settings, but when used in the control of potentially pathogenic microorganisms **outside the body**, chemicals are frequently divided into two categories on the basis of the types of surfaces they are applied to. These two categories are antiseptics and disinfectants.

Antiseptics – Chemicals categorized as antiseptics are those used to control potentially pathogenic microorganisms on **living surfaces**. Sepsis means infection, and chemicals designed to prevent sepsis must be mild enough to be used on skin surfaces or mucous membranes without damaging the eukaryotic cells present.

Disinfectants – Chemicals categorized as disinfectants are those used primarily to control potentially pathogenic microorganisms on **non-living surfaces**. Many chemicals used as disinfectants are not safe for use on living surfaces and should not be applied to them.

Although this distinction is handy, it is not always accurate because some types of chemicals are used on both living and non-living surfaces, e.g., alcohols, some halogens and certain detergents. The distinction between antiseptic and disinfectant may be a matter of concentration, with higher concentrations being used only on non-living surfaces.

If they are to be used routinely, chemicals (both antiseptics and disinfectants) are expected to meet certain criteria, including:

1. They should be effective within a reasonable time period.
2. They should not be toxic or hazardous to persons applying them.
3. They should be readily soluble and able to penetrate into small openings, (because that's where the microorganisms are.
4. They should not damage the surfaces or materials they are being applied to.
5. They should be biodegradable within a reasonable period of time.

Although mercury compounds effectively control microorganisms, they are not biodegradable, and tend to accumulate within human tissues causing severe neurological damage. Mercury compounds are used less extensively than they once were.

Although many different types of chemicals are used to control potentially pathogenic microorganisms outside the body, certain groups of chemicals are applied more commonly than others. Some frequently encountered chemical groups are listed below.

1. **Surfactants** – The term surfactant is an abbreviation for surface-active agent, and applies to a group of chemicals that decrease the surface tension of water. Soaps and detergents fall into this category, and are commonly mixed with other chemicals to increase their penetrating abilities. When used in high concentrations, surfactants can also disrupt cell membranes and cause cell lysis.
2. **Halogens** – The elements chlorine, bromine, iodine and fluorine are halogens and are all, powerful oxidizing agents that can inactivate cellular proteins. Chlorine is used most extensively in water treatment and as a disinfectant, while iodine is often used as an antiseptic. Bromine gas is sometimes used as a fumigating agent, but is highly toxic.
3. **Metal Ions** – The ionic forms of heavy metals including copper, silver, zinc, mercury and lead have been used as both antiseptics and disinfectants. These typically interact with proteins rendering them inactive. Silver nitrate was used extensively to prevent damage from sexually transmitted pathogens in the eyes of newborn infants, while mercury compounds were previously used as antiseptics (Merthiolate and mercurochrome) and as antifungal agents on seed corn. Copper sulfate is used to prevent hoof rot in sheep, cattle and other livestock, and lead nitrate is an ingredient in Desitin, an ointment used to prevent diaper rash.
4. **Alkylating agents** – Alkylating agents can cause the addition of methyl or ethyl groups to organic compounds, and tend to disrupt protein function. Ethylene oxide is an alkylating agent most commonly used as a fumigant.
5. **Formaldehyde** – Formaldehyde reacts with chemical groups (carboxyl, amino and sulfhydryl) and so tends to disrupt protein function.
6. **Alcohols** – Alcohols including ethanol and isopropanol are commonly used as both disinfectants and antiseptics. When used in high concentrations they denature cellular proteins (cause coagulation) and kill most types of cells.
7. **Phenol derivatives** – Phenol, also called carbolic acid, was used by Joseph Lister as a means of preventing sepsis. When used in high concentrations it disrupts cellular membranes and kills cells. Phenol is found in Lysol and Hexachlorophene.
8. **Hydrogen peroxide** – Hydrogen peroxide is a powerful oxidizing agent effective against catalase-negative organisms., i.e., those lacking the ability to produce catalase enzymes. It is often used as an antiseptic and sometimes as a mouth rinse.

When used in high concentrations, chemicals such as those listed above are generally bactericidal; however, their effectiveness can be decreased by accumulations of organic material (debris) and by biofilms. **Biofilms** are formed by populations of bacteria growing on the surfaces of objects and coating themselves with polysaccharide (glycocalyx). The plaque forming on tooth surfaces and the slime common on river rocks are two examples of biofilms. Biofilms often form on catheters, inside tubing, and on other wet surfaces present in clinical settings. When present, they can significantly reduce the effectiveness of chemical control agents.

Antimicrobial Chemotherapy

Chemicals used to control potentially pathogenic microorganisms systemically, i.e., inside the body, are called **antimicrobial drugs** or **chemotherapeutic agents**. In order to be effective, these chemicals must be able to control pathogens without doing damage to host cells or tissue (recall the "magic bullet" concept popularized by Paul Ehrlich during the early 1900s). Although many types of chemicals can be used to control microorganisms outside the body, those available for controlling potential pathogens inside the body are somewhat limited. Antimicrobial drugs can be divided into categories based on the types of pathogens they control, e.g., anti-fungal drugs, anti-viral drugs, anti-helminth drugs, anti-parasitic drugs and antibiotics.

Antibiotics – Antibiotics are antimicrobial agents originally produced by some type of living organism, and used to control bacteria. Most antibiotics were originally produced by bacteria or fungi, but recently chemicals with antibiotic activity have been found in association with multicellular organisms including trees and frogs.

Differential toxicity – The ability of a chemical to control pathogens inside the body without doing damage to host cells or tissues is referred to as **differential toxicity**, and is influenced primarily by the drug's **concentration** and its **mechanism of action**.

The concentration of a chemotherapeutic agent necessary to provide clinical control of a pathogen is called the **therapeutic dose** for that drug. Since many drugs are metabolized, or actively excreted by the body, and since human physiology is variable between patients, there is no single concentration capable of providing clinical control in all individuals. Instead, there is a **therapeutic range** within which a drug can be expected to be effective. The concentration at which drugs are applied must be carefully monitored because if the concentration is too low, the drug will be ineffective, and if it is too high, the drug may have toxic side effects or cause damage within the body.

The lowest concentration of an antimicrobial drug that will effectively inhibit the growth of a specific type of microorganism in vitro is called the **minimal inhibitory concentration** (MIC) and can be determined by a variety of methods, one of which will be demonstrated in the laboratory. Knowing the MICs for specific drugs and their targets can significantly improve the ability of clinicians to effectively treat and cure their patients.

Antimicrobial drugs vary significantly with respect to their mechanisms of action, and it is not uncommon for drugs to have more than one effect on pathogens. Differential toxicity is most readily maintained if drugs act on cellular features unique to prokaryotes, because then they are less likely to damage human cells. Some example mechanisms of action and some specific drug types displaying these actions, are listed below.

1. **Inhibition of enzymatic pathways** – Some types of antimicrobial drugs exert their influence by inhibiting metabolic pathways unique to prokaryotic cells. For example, the **Sulfa drugs** act as **competitive inhibitors**, blocking the activity of enzymes involved in the conversion of para-aminobenzoic acid (PABA) into folic acid, a compound essential for growth. Since human cells cannot form folic acid, the sulfa drugs have good differential toxicity. Sulfa drugs are **static** antimicrobial drugs (but not antibiotics), and some types of bacteria have developed resistance to them.

2. **Inhibition of cell wall synthesis** – Antimicrobial drugs collectively referred to as **β-lactam** antibiotics (Penicillins and Cephalosporins) prevent the formation of peptidoglycan, and so effectively inhibit cell wall synthesis. These drugs are **bactericidal** to actively growing cells because growing cells partially degrade their peptidoglycan walls during elongation, and if they cannot replace this material they die. Since human cells do not form peptidoglycan walls, drugs with this activity have good differential toxicity.

   Penicillins and Cephalosporins are produced by fungi in the genera *Penicillium* and *Cephalosporium* respectively. Although initially very effective in controlling pathogens, they are now losing effectiveness because many bacteria have developed resistance to them by acquiring genes encoding enzymes that degrade them e.g., penicillinases or cephalosporinases (collectively called **β-lactamase** enzymes).

3. **Inhibition of Protein synthesis –** Several different types of antibiotics control pathogens by inhibiting protein synthesis, but not all of them exert their influence in the same manner. **Tetracyclines**, antibiotics produced by bacteria in the genus *Streptomyces*, inhibit translation by preventing the binding of aminoacyl-tRNA molecules to ribosomes. Since these drugs only prevent protein synthesis while present in effective concentrations, their action is not permanent, and they are static rather than cidal. When used to treat young children, they can cause permentent discoloration (darking) of the teeth. Other complications can include liver damage, skin photosensitivity and damage to 30S ribosomal subunits within mitochondria.

   Antibiotics called **Aminoglycosides** also inhibit protein synthesis, but bind permanently to ribosomes (either the 30S or 50S subunits) and block the transfer of peptidyl-t-RNA molecules from the A-site to the P-site. Though their exact mechanism of action is uncertain, the effect is permanent and so these drugs are cidal, i.e., cause cells to die. Aminoglycosides including Streptomycin, neomycin and kanamycin were originally produced by bacteria in the genus *Streptomyces*, while Gentamicin, Tobramicin and Amikacin were made by a bacteria in the genus *Micromonospora.*

   Aminoglycosides can sometimes cause damage to kidneys, so their concentrations and effects must be carefully monitored.

4. **Inhibition of membrane function –** Drugs that interfere with membrane function may act either on the cell membrane or on the outer membrane of the Gram-negative cell wall. Two drugs that interfere with membrane function are **Bacitracin** and **Polymyxin**, antibiotics produced by bacteria in the genus *Bacillus*. Bacitracin inhibits the formation of peptidoglycan by acting on membrane transporters involved in moving materials required for wall synthesis across the cell membrane. Polymyxins interact with phospholipids and disrupt membrane structure (as well as function). Both of these antibiotics are cidal.

5. **Inhibition of nucleic acid synthesis (m-RNA synthesis) –** Two chemicals that inhibit nucleic acid synthesis are Rifampin (Rifampicin) and Actinomycin-D. Rifampin is an antibiotic produced by bacteria in the genus *Streptomyces*, and binds with the beta-subunit of prokaryotic RNA-polymerase, inhibiting transcription. It can have adverse effects on liver tissue. Actinomycin-D, also called Dactinomycin, binds to DNA molecules and inhibits both replication and transcription. Because it is highly toxic to

mammalian cells as well as bacteria, it is used primarily as a chemotherapeutic agent in the treatment of certain cancers.

Antimicrobial drugs are sometimes referred to as narrow-spectrum or broad-spectrum depending on their range of effectiveness. **Narrow-spectrum drugs** effectively control only a few or sometimes only one type of pathogen while **broad-spectrum drugs** control many types (usually both Gram-positive and Gram-negative forms). Formerly, broad-spectrum drugs were preferred because physicians did not appreciate the role played by normal flora. Currently, some attempt is being made to limit treatments to narrow spectrum drugs and to develop drugs with more specific mechanisms of action. This would help prevent damage to bacteria beneficial to the body, and would also help reduce the development of drug-resistant strains.

Not surprisingly, antibiotics are not effective against viruses because viruses do not have metabolic pathways, do not form cell walls, do not synthesize proteins and do not have cell membranes. Antiviral drugs typically act to inhibit the binding of viruses with their host cells, or to inhibit viral replication within cells.