PHYSICAL AGENTS TO CONTROL MICROORGANISMS

A. INTRODUCTION TO THE CONTROL OF MICROORGANISMS

Control of microorganisms is essential in order to prevent the transmission of diseases and infection, stop decomposition and spoilage, and prevent unwanted microbial contamination.

Microorganisms are controlled by means of physical agents and chemical agents. Physical agents include such methods of control as high or low temperature, desiccation, osmotic pressure, radiation, and filtration. Control by chemical agents refers to the use of disinfectants, antiseptics, antibiotics, and chemotherapeutic antimicrobial chemicals.

Basic terms used in discussing the control of microorganisms include:

1. Sterilization
Sterilization is the process of destroying all living organisms and viruses. A sterile object is one free of all life forms, including bacterial endospores, as well as viruses.

2. Disinfection
Disinfection is the elimination of microorganisms from inanimate objects or surfaces.

3. Decontamination
Decontamination is the treatment of an object or inanimate surface to make it safe to handle.\

3. Disinfectant
A disinfectant is an agents used to disinfect inanimate objects but generally to toxic to use on human tissues.

4. Antiseptic
An antiseptic is an agent that kills or inhibits growth of microbes but is safe to use on human tissue.

6. Sanitizer
A sanitizer is an agent that reduces, but may not eliminate, microbial numbers to a safe level.

7. Cidal
An agent that is cidal in action will kill microorganisms and viruses.

8. Static
An agent that is static in action will inhibit the growth of microorganisms.

Keep in mind that when evaluating or choosing a method of controlling microorganisms, you must consider the following factors which may influence antimicrobial activity:

1. the concentration and kind of a chemical agent used;

2. the intensity and nature of a physical agent used;

3. the length of exposure to the agent;

4. the temperature at which the agent is used;

5. the number of microorganisms present;

6. the organism itself; and

7. the nature of the material bearing the microorganism.

B. TEMPERATURE

Microorganisms have a minimum, an optimum, and a maximum temperature for growth. Temperatures **below the minimum** usually have a **static** action on microorganisms. They inhibit microbial growth by slowing down metabolism but do not necessarily kill the organism. Temperatures **above the maximum** usually have a **cidal** action, since they denature microbial enzymes and other proteins. Temperature is a very common and effective way of controlling microorganisms.

1. High Temperature

Vegetative microorganisms can generally be killed at temperatures from 50°C to 70°C with moist heat. Bacterial **endospores**, however, are very resistant to heat and extended exposure to much higher temperature is necessary for their destruction. High temperature may be applied as either moist heat or dry heat.

a. Moist heat

Moist heat is generally more effective than dry heat for killing microorganisms because of its ability to **penetrate** microbial cells. Moist heat kills microorganisms by **denaturing their proteins** (causes proteins and enzymes to lose their three-dimensional functional shape). It also may **melt lipids** in cytoplasmic membranes.

1. Autoclaving

Autoclaving employs **steam under pressure**. Water normally boils at 100°C; however, when put under pressure, water boils at a higher temperature. During autoclaving, the materials to be sterilized are placed under **15 pounds per square inch of pressure** in a pressure-cooker type of apparatus. When placed under 15 pounds of pressure, the boiling point of water is raised to **121°C**, a temperature sufficient to kill bacterial endospores.

The time the material is left in the autoclave varies with the nature and amount of material being sterilized. Given sufficient time (generally 15-45 minutes), autoclaving is **cidal** for both vegetative organisms and endospores, and is the most common method of sterilization for materials not damaged by heat.

2. Boiling water

Boiling water (100°C) will generally kill vegetative cells after about 10 minutes of exposure. However, certain viruses, such as the hepatitis viruses, may survive exposure to boiling water for up to 30 minutes, and endospores of certain *Clostridium* and *Bacillus* species may survive even hours of boiling.

b. Dry heat

Dry heat kills microorganisms through a process of **protein oxidation** rather than protein coagulation. Examples of dry heat include:

1. Hot air sterilization

Microbiological ovens employ very high dry temperatures: 171°C for 1 hour; 160°C for 2 hours or longer; or 121°C for 16 hours or longer depending on the volume. They are generally used only for sterilizing glassware, metal instruments, and other inert materials like oils and powders that are not damaged by excessive temperature.

2. **Incineration**

Incinerators are used to destroy disposable or expendable materials by burning. We also sterilize our inoculating loops by incineration.

c. **Pasteurization**

Pasteurization is the mild heating of milk and other materials to kill **particular spoilage organisms or pathogens**. It does not, however, kill all organisms. Milk is usually pasteurized by heating to 71.6°C for at least 15 seconds in the flash method or 62.9°C for 30 minutes in the holding method.

2. **Low Temperature**

Low temperature **inhibits** microbial growth by **slowing down microbial metabolism**. Examples include refrigeration and freezing. Refrigeration at 5°C slows the growth of microorganisms and keeps food fresh for a few days. Freezing at -10°C stops microbial growth, but generally does not kill microorganisms, and keeps food fresh for several months.

C. DESICCATION

Desiccation, or drying, generally has a **static** effect on microorganisms. Lack of water inhibits the action of microbial enzymes. Dehydrated and freeze-dried foods, for example, do not require refrigeration because the absence of water inhibits microbial growth.

D. OSMOTIC PRESSURE

Microorganisms, in their natural environments, are constantly faced with alterations in osmotic pressure. Water tends to flow through semipermeable membranes, such as the cytoplasmic membrane of microorganisms, towards the side with a higher concentration of dissolved materials (**solute**). In other words, **water moves from greater water (lower solute) concentration to lesser water (greater solute) concentration**.

When the concentration of dissolved materials or solute is higher inside the cell than it is outside, the cell is said to be in a **hypotonic environment** and water will flow **into the cell**. The rigid **cell walls** of bacteria and fungi, however, **prevent bursting or plasmoptysis**. If the concentration of solute is the same both inside and outside the cell, the cell is said to be in an **isotonic environment**. Water flows equally in and out of the cell. Hypotonic and isotonic environments are not usually harmful to microorganisms. However, if the concentration of dissolved materials or solute is higher outside of the cell than inside, then the cell is in a **hypertonic environment**. Under this condition, water flows **out of the cell**, resulting in shrinkage of the cytoplasmic membrane or **plasmolysis**. Under such conditions, the cell becomes **dehydrated** and its **growth is inhibited**.

The canning of jams or preserves with a high sugar concentration inhibits bacterial growth through hypertonicity. The same effect is obtained by salt-curing meats or placing foods in a salt brine. This **static action** of osmotic pressure thus prevents bacterial decomposition of the food. Molds, on the other hand, are more tolerant of hypertonicity. Foods, such as those mentioned

above, tend to become overgrown with molds unless they are first sealed to exclude oxygen. (Molds are aerobic.)

E. RADIATION

1. Ultraviolet Radiation

The ultraviolet portion of the light spectrum includes all radiations with wavelengths from 100 nm to 400 nm. It has low wave-length and low energy. The microbicidal activity of ultraviolet (UV) light depends on **the length of exposure**: the longer the exposure the greater the cidal activity. It also depends on the **wavelength of UV used**. The most cidal wavelengths of UV light lie in the **260 nm - 270 nm range** where it is absorbed by nucleic acid.

In terms of its mode of action, UV light is absorbed by microbial DNA and causes adjacent thymine bases on the same DNA strand to covalently bond together, forming what are called **thymine-thymine dimers.**
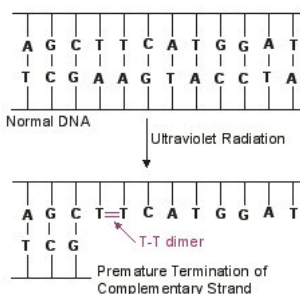
As the DNA replicates, nucleotides do not complementary base pair with the thymine dimers and this terminates the replication of that DNA strand. However, **most of the damage from UV radiation actually comes from the cell trying to repair the damage to the DNA by a process called SOS repair**. In very heavily damaged DNA containing large numbers of thymine dimers, a process called SOS repair is activated as kind of a last ditch effort to repair the DNA. In this process, a gene product of the SOS system binds to DNA polymerase allowing it to synthesize new DNA across the damaged DNA. However, this **altered DNA polymerase loses its proofreading ability** resulting in the synthesis of DNA that itself now contains many misincorporated bases. In other words, **UV radiation causes mutation** and can lead to faulty protein synthesis. With sufficient mutation, bacterial metabolism is blocked and the organism dies. Agents such as UV radiation that cause high rates of mutation are called **mutagens**.

The effect of this inproper base pairing may be reversed to some extent by exposing the bacteria to strong visible light immediately after exposure to the UV light. The visible light activates an enzyme that breaks the bond that joins the thymine bases, thus enabling correct complementary base pairing to again take place. This process is called **photoreactivation**.

UV lights are frequently used to reduce the microbial populations in hospital operating rooms and sinks, aseptic filling rooms of pharmaceutical companies, in microbiological hoods, and in the processing equipment used by the food and dairy industries.

An important consideration when using UV light is that it has **very poor penetrating power**. Only microorganisms on the **surface** of a material that are exposed directly to the radiation are susceptible to destruction. UV light can also damage the eyes, cause burns, and cause mutation in cells of the skin.

2. Ionizing Radiation

Ionizing radiation, such as **X-rays and gamma rays**, has much more energy and penetrating power than ultraviolet radiation. It ionizes water and other molecules to form radicals (molecular fragments with unpaired electrons) that can **disrupt DNA molecules and proteins**. It is often used to sterilize pharmaceuticals and disposable medical supplies such as syringes, surgical

gloves, catheters, sutures, and petri plates. It can also be used to retard spoilage in seafoods, meats, poultry, and fruits.

F. FILTRATION

Microbiological membrane filters provide a useful way of sterilizing materials such as vaccines, antibiotic solutions, animal sera, enzyme solutions, vitamin solutions, and other solutions that may be damaged or denatured by high temperatures or chemical agents. The filters contain pores small enough to prevent the passage of microbes but large enough to allow the organism-free fluid to pass through. The liquid is then collected in a sterile flask.
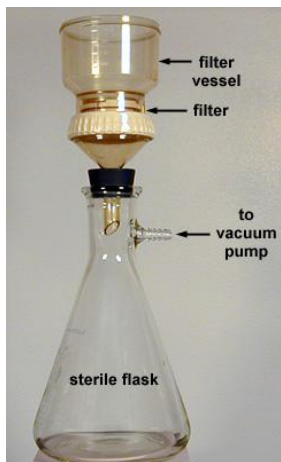
Filters with a pore diameter from 25 nm to 0.45 µm are usually used in this procedure. Filters can also be used to remove microorganisms from water and air for microbiological testing.

BACTERIOLOGIC EXAMINATION OF WATER: COLIFORM COUNTS

The purpose of the bacteriological examination of water is to determine if there is a possibility of pathogens being present. Infectious diseases such as salmonellosis, typhoid fever, shigellosis, cholera, hepatitis A, amoebic dysentery, *Campylobacter* gastroenteritis, giardiasis, and other fecal-oral route diseases may be transmitted by fecally-contaminated water. The identification of pathogens, however, is quite difficult. Pathogens may not survive long in water and are usually present only in small quantity. Therefore, one usually tests for the presence of coliforms in water.

Coliforms are gram-negative, lactose-fermenting rods of the family Enterobacteriaceae. *Escherichia coli*, a fecal coliform, is normal flora of the intestines in humans and animals and is, therefore, a **direct indicator of fecal contamination** of the water. The presence of coliforms would then indicate the possibility of fecal pathogens being present.

Two tests are frequently performed to monitor water: the fecal coliform count and the total coliform count.

1. The **fecal coliform count** tests specifically for the fecal coliform *E. coli*. M-FC medium is used in this test and the plates are incubated at 45.5C. This temperature is **selective for fecal coliforms** (nonfecal coliforms will not grow at this temperature) which produce blue colonies. This test, however, requires a special water bath incubator to assure a temperature of 45.5C.

2. The **total coliform count** will detect **any coliforms (fecal and nonfecal)** present in the water. It is not as specific an indicator of fecal contamination, but is a useful **screening test**. M-coliform medium is used in this test and the plates are incubated at 37C. **Both fecal and nonfecal coliforms will grow and produce metallic green colonies**. Coliforms would indicate the **possibility of fecal contamination of the water**.

Both of these tests use the micropore membrane filter method. Different amounts of the water sample being tested are passed through a membrane filter. The water passes through and the bacteria are trapped on the surface of the filter. The filter is then placed in a petri plate on pads containing either M-FC or M-coliform medium. Colonies then form on the filter. By counting the number of colonies and knowing the volume of water sample used, the number of fecal coliforms or total coliforms per ml of water can be determined.

Questions:

1. Define the following terms: sterilization, disinfection, decontamination, static, and cidal.

2. State whether moist or dry heat is more effective in controlling microorganisms, and indicate why.

3. State specifically how moist heat kills microorganisms.

4. State two methods of applying moist heat.

5. Briefly describe the process of autoclaving (pressure, time, and temperature).

6. State whether or not boiling is an effective means of sterilization, and indicate why.

7. State specifically how dry heat kills microorganisms.

8. State two methods of applying dry heat.

9. Define pasteurization.

10. State whether low temperature has a static or cidal effect on microorganisms, and indicate why.

11. State whether desiccation has a static or a cidal effect on microorganisms, and indicate how it affects the cell.

12. Describe osmosis in terms of water flow through a semipermeable membrane.

13. Define the following terms: hypotonic, hypertonic, isotonic, plasmoptysis, and plasmolysis.

14. State why hypotonicity does not normally harm bacteria.

15. Describe how bacterial growth is inhibited in jams and salt-cured meats.

16. State why jams still must be sealed even though bacteria will not grow in them.

17. State whether hypertonicity has a static or a cidal effect on microorganisms.

18. State how the wavelength and the length of exposure influence the bacteriocidal effect of UV light.

19. Describe specifically how UV light kills microorganisms.

20. State why UV light is only useful as a means of controlling surface contaminants and give several practical applications.

21. Describe how ionizing radiation kills microorganisms and state several common applications.

22. State the concept behind sterilizing solutions with micropore membrane filters.

23. State why filters are preferred over autoclaving for such materials as vaccines, antibiotic solutions, sera, and enzyme solutions.

DISINFECTANTS ANTISEPTICS AND SANITIZERS TO CONTROL MICROORGANISMS

A. DISINFECTANTS, ANTISEPTICS, AND SANITIZERS

Disinfection is the elimination of microorganisms from inanimate objects or surfaces, whereas **decontamination** is the treatment of an object or inanimate surface to make it safe to handle.

a. The term **disinfectant** is used for an agent used to disinfect inanimate objects or surfaces but is generally to toxic to use on human tissues.

b. The term **antiseptic** refers to an agent that kills or inhibits growth of microbes but is safe to use on human tissue.

c. The term **sanitizer** describes an agent that reduces, but may not eliminate, microbial numbers to a safe level.

Because disinfectants and antiseptics often work slowly on some viruses - such as the hepatitis viruses, bacteria with an **acid-fast cell wall** such as *Mycobacterium tuberculosis*, and especially bacterial **endospores**, produced by the genus *Bacillus* and the genus *Clostridium*, they are usually **unreliable for sterilization** - the destruction of **all** life forms.

There are a number of factors which influence the antimicrobial action of disinfectants and antiseptics, including:

1. The **concentration** of the chemical agent.

2. The **temperature** at which the agent is being used. Generally, the lower the temperature, the longer it takes to disinfect or decontaminate.

3. The **kinds of microorganisms** present. Endospore producers such as *Bacillus* species, *Clostridium* species, and acid-fast bacteria like *Mycobacterium tuberculosis* are harder to eliminate.

4. The **number of microorganisms** present. The more microorganisms present, the harder it is to disinfect or decontaminate.

5. The **nature of the material bearing the microorganisms**. Organic material such as dirt and excreta interferes with some agents.

The best results are generally obtained when the initial **microbial numbers are low** and when the **surface to be disinfected is clean** and free of possible interfering substances.

There are 2 common antimicrobial **modes of action** for disinfectants, antiseptics, and sanitizers:

1. They may **damage the lipids and/or proteins of the semipermeable cytoplasmic membrane** of microorganisms resulting in **leakage of cellular materials** needed to sustain life.

2. They may **denature microbial enzymes and other proteins,** usually by disrupting the hydrogen and disulfide bonds that give the protein its three-dimensional functional shape. This **blocks metabolism**.

A large number of such chemical agents are in common use. Some of the more common groups are listed below:

1. **Phenol and phenol derivatives**

Phenol (5-10%) was the first disinfectant commonly used. However, because of its toxicity and odor, phenol derivatives are now generally used. These include orthophenylphenol, hexachlorophene, triclosan, hexylresorcinol, and chlorhexidine. Orthophenylphenol is the agent in Lysol®, O-syl®, Staphene®, and Amphyl®. Hexachlorophene in a 3% solution is combined with detergent and is found in PhisoHex®. Triclosan is a chlorine-containing phenolic antiseptic very common in antimicrobial soaps and other products. Hexylresorcinol is in throat lozenges and ST-37. A 4% solution of chlorhexidine in isopropyl alcohol and combined with detergent (Hibiclens® and Hibitane®) is a common handwashing agent and surgical handscrub. These agents kill most bacteria, most fungi, and some viruses, but are usually ineffective against endospores. They **alter membrane permeability and denature proteins**.

2. **Soaps and detergents**

Soaps are only mildly microbicidal. Their use aids in the **mechanical removal** of microorganisms by breaking up the oily film on the skin (emulsification) and reducing the surface tension of water so it spreads and penetrates more readily. Some cosmetic soaps contain added antiseptics to increase antimicrobial activity.

Detergents may be anionic or cationic. **Anionic** (negatively charged) detergents, such as laundry powders, **mechanically remove microorganisms and other materials** but are not very microbicidal. **Cationic** (positively charged) detergents **alter membrane permeability and denature proteins**. They are effective against many vegetative bacteria, some fungi, and some viruses. However, bacterial endospores and certain bacteria such as *Mycobacterium tuberculosis* and *Pseudomonas* species are usually resistant. They are also inactivated by soaps and organic materials like excreta. Cationic detergents include the quaternary ammonium compounds such as benzalkonium chloride, zephiran, diaprene, roccal, ceepryn, and phemerol.

3. **Alcohols**

70% solutions of ethyl or isopropyl alcohol are effective in killing vegetative bacteria, enveloped viruses, and fungi. However, they are usually ineffective against endospores and non-enveloped viruses. Once they evaporate, their cidal activity will cease. Alcohols **denature membranes** and are often combined with other disinfectants, such as iodine, mercurials, and cationic detergents for increased effectiveness.

4. **Acids and alkalies**

Acids and alkalies **alter membrane permeability and denature proteins and other molecules**. Salts of **organic acids**, such as calcium propionate, potassium sorbate, and methylparaben, are commonly used as food preservatives. Undecylenic acid (Desenex®) is used for dermatophyte infections of the skin. An example of an **alkali** is lye (sodium hydroxide).

5. Heavy metals

Heavy metals, such as mercury, silver, and copper, **denature proteins**. Mercury compounds (mercurochrome, metaphen, merthiolate) are only bacteriostatic and are not effective against endospores. Silver nitrate (1%) is sometimes put in the eyes of newborns to prevent gonococcal ophthalmia. Copper sulfate is used to combat fungal diseases of plants and is also a common algicide. Selinium sulfide kills fungi and their spores.

6. Chlorine

Chlorine gas reacts with water to form **hypochlorite ions**, which in turn **denature microbial enzymes**. Chlorine is used in the chlorination of drinking water, swimming pools, and sewage. Sodium hypochlorite is the active agent in household bleach. Calcium hypochlorite, sodium hypochlorite, and chloramines (chlorine plus ammonia) are used to sanitize glassware, eating utensils, dairy and food processing equipment, hemodialysis systems, and treating water supplies.

7. Iodine and iodophores

Iodine also **denatures microbial proteins**. Iodine tincture contasns a 2% solution of iodine and sodium iodide in 70% alcohole. Aqueous iodine solutions containing 2% iodine and 2.4% sodium iodide are commonly used as a topical antiseptic. **Iodophores** are a combination of iodine and an inert polymers such as polyvinylpyrrolidone that reduces surface tension and slowly releases the iodine. Iodophores are less irritating than iodine and do not stain. They are generally effective against vegetative bacteria, *Mycobacterium tuberculosis*, fungi, some viruses, and some endospores. Examples include Wescodyne®, Ioprep®, Ioclide®, Betadine®, and Isodine®.

8. Aldehydes.
Aldehydes, such as formaldehyde and glutaraldehyde, **denature microbial proteins**. Formalin (37% aqueous solution of formaldehyde gas) is extremely active and kills most forms of microbial life. It is used in embalming, preserving biological specimens, and in preparing vaccines. Alkaline glutaraldehyde (Cidex®), acid glutaraldehyde (Sonacide®), and glutaraldehyde phenate solutions (Sporocidin®) kill vegetative bacteria in 10-30 minutes and endospores in about 4 hours. A 10 hour exposure to a **2% glutaraldehyde** solution can be used for **cold sterilization** of materials.

9. Ethylene oxide gas

Ethylene oxide is one of the very few chemicals that can be relied upon for **sterilization** (after 4-12 hours exposure). Since it is explosive, it is usually mixed with inert gases such as freon or carbon dioxide. **Gaseous chemosterilizers**, using ethylene oxide, are commonly used to sterilize heat-sensitive items such as plastic syringes, petri plates, textiles, sutures, artificial heart valves, heart-lung machines, and mattresses. Ethylene oxide has very high penetrating power and **denatures microbial proteins**. Vapors are toxic to the skin, eyes, and mucous membranes and are also carcinogenic. Another gas that is used as a sterilant is **chlorine dioxide** which denatures proteins in vegetative bacteria, bacterial endospores, viruses, and fungi.

B. EVALUATION OF DISINFECTANTS, ANTISEPTICS, AND SANITIZERS

It is possible to evaluate disinfectants, antiseptics, and sanitizers using either in vitro or in vivo tests. An *in vitro* test is one done under **artificial, controlled laboratory conditions**. An *in vivo* test is one done under the **actual conditions of normal use**.

A common *in vitro* test is to compare the antimicrobial activity of the agent being tested with that of phenol. The resulting value is called a phenol coefficient and has some value in comparing the strength of disinfectants under standard conditions. Phenol coefficients may be misleading, however, because as mentioned earlier, the killing rate varies greatly with the conditions under which the chemical agents are used. The concentration of the agent, the temperature at which it is being used, the length of exposure to the agent, the number and kinds of microorganisms present, and the nature of the material bearing the microorganisms all influence the antimicrobial activity of a disinfectant. If a disinfectant is being evaluated for possible use in a given *in vivo* situation, it must be **evaluated under the same conditions in which it will actually be used**.

C. EFFECTIVENESS OF HAND WASHING

There are 2 categories of microorganisms, or flora, normally found on the hands. **Resident flora** are the normal flora of the skin. **Transient flora** are the microorganisms you pick up from what you have been handling. It is routine practice to wash the hands prior to and after examining a patient and to do a complete regimented surgical scrub prior to going into the operating room. This is done in order to remove the potentially harmful transient flora, reduce the number of resident flora, and disinfect the skin.

Actual sterilization of the hands is not possible since microorganisms live not only on the surface of the skin but also in deeper skin layers, in ducts of sweat glands, and around hair follicles. These normal flora are mainly nonpathogenic staphylococci and diphtheroid bacilli.

Questions:

1. Define the following terms: sterilization, disinfection, decontamination, disinfectant, antiseptic, and sanitizer.

2. State why chemical agents are usually unreliable for sterilization.

3. List five factors that may influence the antimicrobial action of disinfectants, antiseptics, and sanitizers.

4. Describe two modes of action of disinfectants, antiseptics, and sanitizers, i.e., how they harm the microorganisms.

5. Name two chemical agents that are reliable for sterilization.

6. Define transient flora and resident flora and compare the two groups in terms of ease of removal.