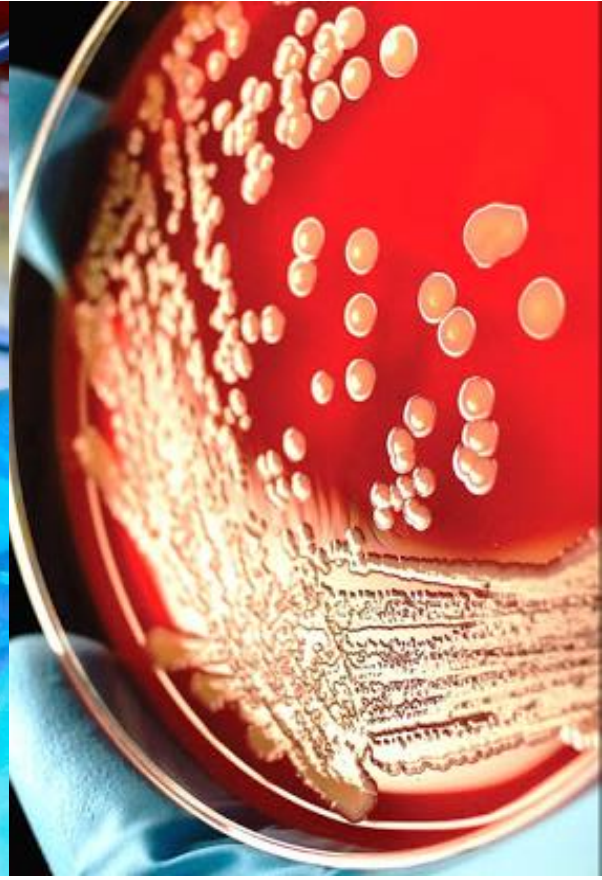

# C-2: Bacteriology

## Unit 4 – Growth and nutrition





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# The common nutrient requirements

- **Macroelements or macronutrients**: required by microorganisms in relatively large amounts.
- **Carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus**: components of carbohydrates, lipids, proteins, and nucleic acids.
- **Potassium, calcium, magnesium, and iron**: exist in the cell as cations and play a variety of roles.
- Micronutrients or trace elements: The **micronutrients—manganese, zinc, cobalt, molybdenum, nickel, and copper—** are needed by most cells





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# Nutritional types of microorganisms

- **Carbon Sources**
- **Autotrophs** : $CO_2$ sole or principal biosynthetic carbon Source
- **Heterotrophs**: Reduced, preformed, organic molecules from other organisms

- **Energy Sources**
- **Phototrophs**: light as their energy source
- **Chemotrophs**: obtain energy from the oxidation of chemical compounds (either organic or inorganic)

- **Electron Sources**
- **Lithotrophs**: use reduced inorganic substances as electron source.
- **Organotrophs**: extract electrons from reduced organic compounds.





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# Major nutritional types of microorganisms

| Nutritional Type | Carbon Source | Energy Source | Electron Source | Representative Microorganisms |
|---|---|---|---|---|
| Photolithoautotrophy (photolithotrophic autotrophy) | $CO_2$ | Light | Inorganic $e^-$ donor | Purple and green sulfur bacteria, cyanobacteria |
| Photoorganoheterotrophy (photoorganotrophic heterotrophy) | Organic carbon, but $CO_2$ may also be used | Light | Organic $e^-$ donor | Purple nonsulfur bacteria, green nonsulfur bacteria |
| Chemolithoautotrophy (chemolithotrophic autotrophy) | $CO_2$ | Inorganic chemicals | Inorganic $e^-$ donor | Sulfur-oxidizing bacteria, hydrogen-oxidizing bacteria, methanogens, nitrifying bacteria, iron-oxidizing bacteria |
| Chemolithoheterotrophy or mixotrophy (chemolithotrophic heterotrophy) | Organic carbon, but $CO_2$ may also be used | Inorganic chemicals | Inorganic $e^-$ donor | Some sulfur-oxidizing bacteria (e.g., *Beggiatoa*) |
| Chemoorganoheterotrophy (chemoorganotrophic heterotrophy) | Organic carbon | Organic chemicals often same as C source | Organic $e^-$ donor, often same as C source | Most nonphotosynthetic microbes, including most pathogens, fungi, many protists, and many archaea |



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# Culture medium

- A **culture medium** is a solid or liquid preparation used to **grow, transport, and store microorganisms**. To be effective, the medium must contain all the nutrients the microorganism requires for growth.
- Microbes that are introduced into a culture medium to initiate growth are called an **inoculum**.
- The microbes that grow and multiply in or on a culture medium are referred to as a **culture**.

***A single colony can contain more than 10 million ($10^7$) individual cells.***





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# Components of media

Any microbial medium should include sources of **nutrition, energy, essential minerals, buffering agents and pH indicators**. Sometimes they also include selective agents and solidifying agents. The following are the essential components of a typical microbial medium:

**Nutrition sources:** sources of proteins, vitamins, mineral and carbohydrates; mostly mixture of peptone and meat extract

**Energy sources:** sources of carbohydrates; mostly glucose

**Essential minerals:** sources of micro and macro minerals; mostly present in peptone and meat extract

**Buffering agents:** agents that maintain optimum pH; mostly specific amino acids, phosphates, citrates and zwitterions

**pH indicators:** agents that indicate variation in pH of the medium by change in color; mostly phenol red

**Selective agents:** agents that allow the growth of only specific bacteria; antibiotics, azides and bile salts are examples

**Solidifying agents:** agents that maintain the medium in solid or semi-solid state, if required; widely used agent is agar though gelatin is sometimes used

Specific substances such as growth factors, enzymes may be incorporated into the medium for specific bacteria

# Basic media

- These are simple media used to support the growth of microorganisms that do not have special nutritional requirements. They include **nutrient broth**, **peptone water** and **nutrient agar**.



**nutrient broth**

**peptone water**

**nutrient agar**



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# Synthetic media

**Synthetic medium** or **defined media** are prepared by adding precise amounts of highly purified inorganic or organic chemicals to distilled water. Therefore, the **exact composition of a defined medium (in both a qualitative and quantitative sense) is known**.

**Table 7.5 Examples of Defined Media**

| BG–11 Medium for Cyanobacteria | Amount (g/liter) | Medium for *Escherichia coli* | Amount (g/liter) |
|---|---|---|---|
| $NaNO_3$ | 1.5 | Glucose | 1.0 |
| $K_2HPO_4 \cdot 3H_2O$ | 0.04 | $Na_2HPO_4$ | 16.4 |
| $MgSO_4 \cdot 7H_2O$ | 0.075 | $KH_2PO_4$ | 1.5 |
| $CaCl_2 \cdot 2H_2O$ | 0.036 | $(NH_4)_2SO_4$ | 2.0 |
| Citric acid | 0.006 | $MgSO_4 \cdot 7H_2O$ | 200.0 mg |
| Ferric ammonium citrate | 0.006 | $CaCl_2$ | 10.0 mg |
| EDTA ($Na_2Mg$ salt) | 0.001 | $FeSO_4 \cdot 7H_2O$ | 0.5 mg |
| $Na_2CO_3$ | 0.02 | Final pH 6.8–7.0 | |
| Trace metal solution[1] | 1.0 ml/liter | | |
| Final pH 7.4 | | | |

# Complex media

- Media that contain **some ingredients of unknown chemical** composition are **complex media**. They are very useful because a single complex medium may be able to meet all the nutritional requirements of **many different microorganisms**.

- These digests are commercially available in dehydrated form and can be easily prepared. However, the **disadvantage of a complex medium is its imprecise nutritional composition**.

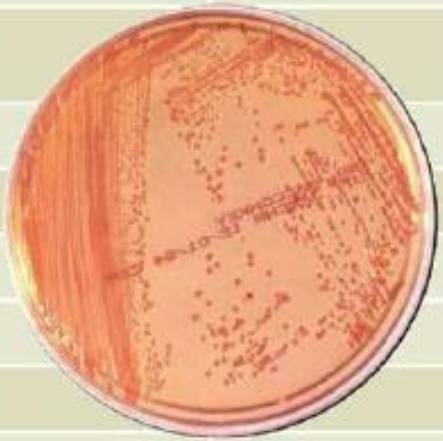
- Complex media employ digests of microbial, animal or plant products, such as **casein (milk protein), beef (beef extract), soybeans (tryptic soy broth)**, yeast cells (yeast extract), or any of a number of other highly nutritious yet impure substances.

# Examples of Complex media



**Table 7.6 Some Common Complex Media**

| Nutrient Broth | Amount (g/liter) | Tryptic Soy Broth | Amount (g/liter) | MacConkey Agar | Amount (g/liter) |
|---|---|---|---|---|---|
| Peptone (gelatin hydrolysate) | 5 | Tryptone (pancreatic digest of casein) | 17 | Pancreatic digest of gelatin | 17.0 |
| Beef extract | 3 | Peptone (soybean digest) | 3 | Pancreatic digest of casein | 1.5 |
| | | Glucose | 2.5 | Peptic digest of animal tissue | 1.5 |
| | | Sodium chloride | 5 | Lactose | 10.0 |
| | | Dipotassium phosphate | 2.5 | Bile salts | 1.5 |
| | | | | Sodium chloride | 5.0 |
| | | | | Neutral red | 0.03 |
| | | | | Crystal violet | 0.001 |
| | | | | Agar | 13.5 |



MacConkey Agar



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# Enriched media

- An **enriched media** contains complex organic substances such as blood, serum, hemoglobin, or special growth factors that must be provided to **certain species in order for them to grow**. An enriched medium, often used for the culture of otherwise difficult-to-grow nutritionally demanding (fastidious) microorganisms.

- Examples of enriched media include **sheep blood agar and chocolate (heated blood) agar**. Selective media contain ingredients that inhibit the growth of some organisms but allow others to grow.

- Enrichment cultures are usually the first step in isolating pure cultures. They use selective conditions (media, environmental conditions) to promote the growth of certain microbes. After enrichment, pure cultures usually are obtained by isolating individual cells with any of three plating techniques: the streak-plate, spread-plate, and pour-plate methods.





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# Enrichment culture

- Enrichment culture techniques are based on recreating a microenvironment in the laboratory to allow abundant growth of a microorganism formerly restricted to a small ecological niche.

- At the same time, the growth of other microbial types is inhibited. This approach often plays a central role in finding new microbes. The success of an enrichment culture depends on a thorough understanding of the specific niche the microbe of interest inhabits and the physiological features that set that microbe apart from others.

- Enrichment cultures are used to promote the growth of one type of microbe, while suppressing the growth of others. This is accomplished by designing media and culture conditions that favor the growth of the desired microorganisms but limit the growth of others.

# Differential media

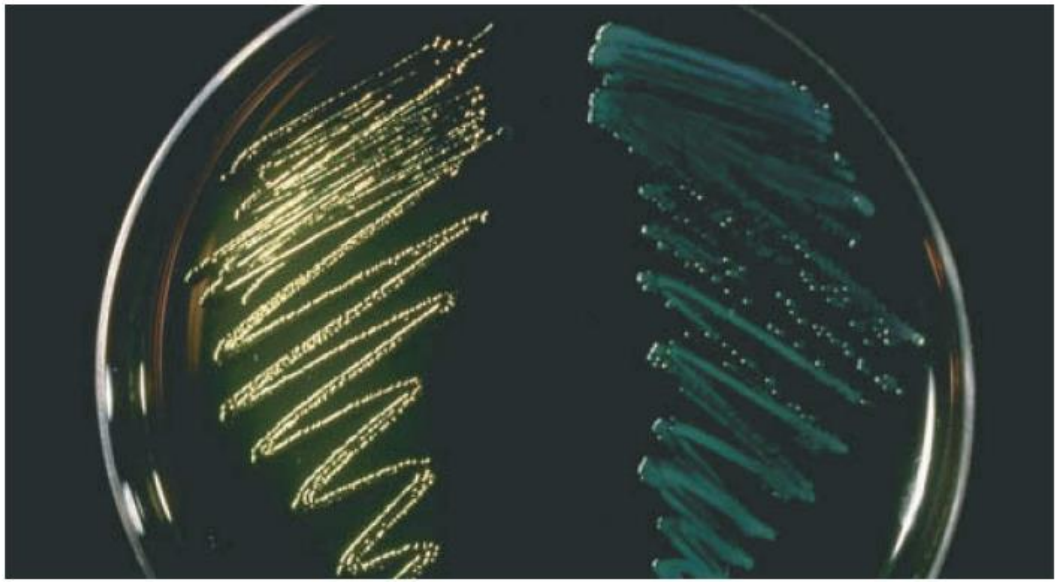
- A **differential medium** is one in which an indicator, typically a reactive dye, is added that reveals whether a particular chemical reaction has occurred during growth. **Differential media are quite useful for distinguishing different species of bacteria** and are therefore widely used in clinical diagnostics and systematic microbiology.

- Examples of differential media include: **Blood agar**, that contains bovine heart blood that becomes transparent in the presence of hemolytic. **Streptococcuseosin methylene blue** (EMB), which is differential for lactose and sucrose fermentation.

# Selective media

A **selective media** contains compounds that inhibit the growth of some microorganisms but not others. Eosin methylene blue agar and MacConkey agar are widely used for the detection of *E. coli* and related bacteria in water supplies and elsewhere. These media suppress the growth of Gram-positive bacteria.





***Escherichia coli*, a lactose fermenter (left), and *Pseudomonas aeruginosa*, a non–lactose fermenter (right) growing on eosin–methylene blue (EMB) agar**



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# Blood agar

- Blood agar is both a **differential medium** and **an enriched one**. It distinguishes between hemolytic and nonhemolytic bacteria.

- Some hemolytic bacteria (e.g., many streptococci and staphylococci isolated from throats) produce clear zones around their colonies because of red blood cell destruction.

- Blood agar is an **enriched growth medium** in that blood (usually sheep blood) provides protein, carbohydrate, lipid, iron, and a number of growth factors and vitamins necessary for the cultivation of fastidious organisms.

- Blood Agar is used to grow a wide range of pathogens particularly those that are more difficult to grow such as *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria* species.





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- **Alpha hemolysis** is defined by a greenish-grey or brownish discoloration around the colony as a result of the **partial lysis of the red blood cells**.
- **Beta hemolysis** is defined by a clear zone of hemolysis under and around the colonies when grown on blood agar. The clear zone appears as a result of the **complete lysis of the red blood cells** present in the medium.
- **Gamma hemolysis** is also called **non-hemolysis as no lysis of red blood** cells occurs. As a result, no change of coloration or no zone of hemolysis was observed under or around the colonies.


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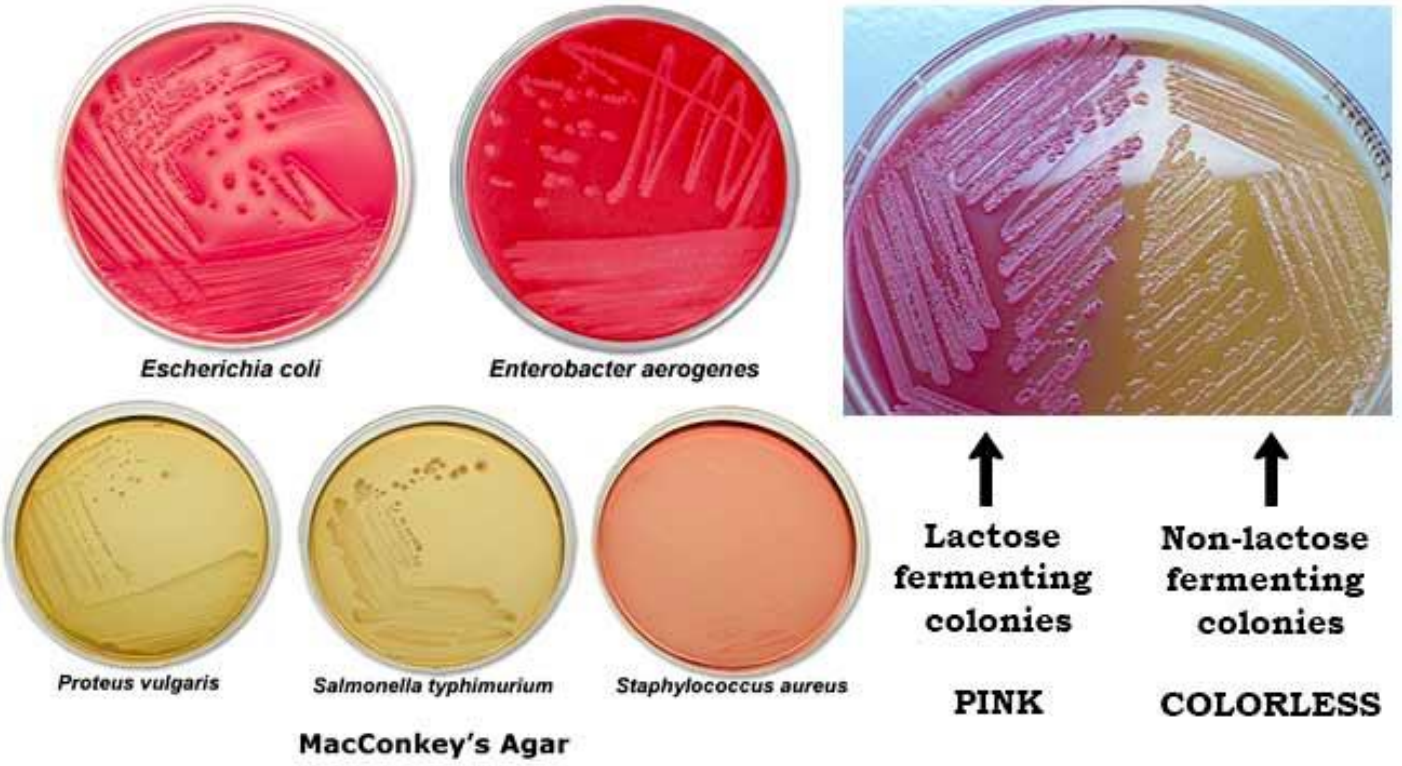
# MacConkey agar (MAC)

- MacConkey agar is both **differential** and **selective**. Because it contains lactose and neutral red dye, bacteria that catabolize lactose by fermenting it release acidic waste products that make colonies **appear pink to red in color.** These are easily distinguished from colonies of bacteria that do not ferment lactose.





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# MacConkey agar (MAC)

- MacConkey agar is used for the isolation of **gram-negative enteric bacteria** and the **differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria**.

- **Pancreatic digest of gelatin** and **peptones (meat and casein)** provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms.

- **Lactose monohydrate** is the fermentable source of carbohydrate. The selective action of this medium is attributed to **crystal violet** and **bile salts,** which are inhibitory to most species of gram-positive bacteria.

- **Sodium chloride** maintains the osmotic balance in the medium. **Neutral red is a pH indicator** that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8.





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# Solidifying agent: Agar

- Solidified media are particularly important because they can be used to isolate different microbes from each other to establish pure cultures.

- **Agar** is the most commonly used solidifying agent. It is a sulfated polymer composed mainly of **D-galactose, 3,6-anhydro-L-galactose, and D-glucuronic acid**.

- It usually is extracted from **red algae**. Agar is well suited as a solidifying agent for several reasons. One is that it melts at about 90°C but, once melted, does not harden until it reaches about 45°C. Thus after being melted in boiling water, it can be cooled to a temperature that is **tolerated by human hands as well as microbes**. Furthermore, microbes growing on agar medium can be incubated at a **wide range of temperatures**. Finally, agar is an excellent hardening agent because most microorganisms **cannot degrade it**.





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# A Comparison of Special Culture Media

| Name | Components | Uses | Examples |
|---|---|---|---|
| Selective medium | Growth stimulants<br>Growth inhibitors | Selecting certain microbes out of a mixture | Mannitol salt agar for staphylococci |
| Differential medium | Dyes<br>Growth stimulants<br>Growth inhibitors | Distinguishing different microbes in a mixture | MacConkey agar for gram-negative bacteria |
| Enriched medium | Growth stimulants | Cultivating fastidious microbes | Blood agar for streptococci; chocolate agar for *Neisseria* species |





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# VBNC (viable but non-culturable) state

- Many of the Bacteria and Archaea are impossible to cultivate in any laboratory culture medium yet devised. Such nonculturable organisms are said to be in a **VBNC (viable but non-culturable) state**. Procedures for identifying VBNC organisms include direct microscopic examination and amplification of diagnostic gene sequences or 16S rRNA sequences.

- Microbiologists believe that part of the reason may be due to their presence in a "foreign" environment because most species have adapted to their own familiar and specific environment; a complex or synthetic medium is not their typical home. Therefore, these species go into a type of dormancy state and do not divide; that is, they are viable, but not culturable.

# Physical and chemical methods of microbial control

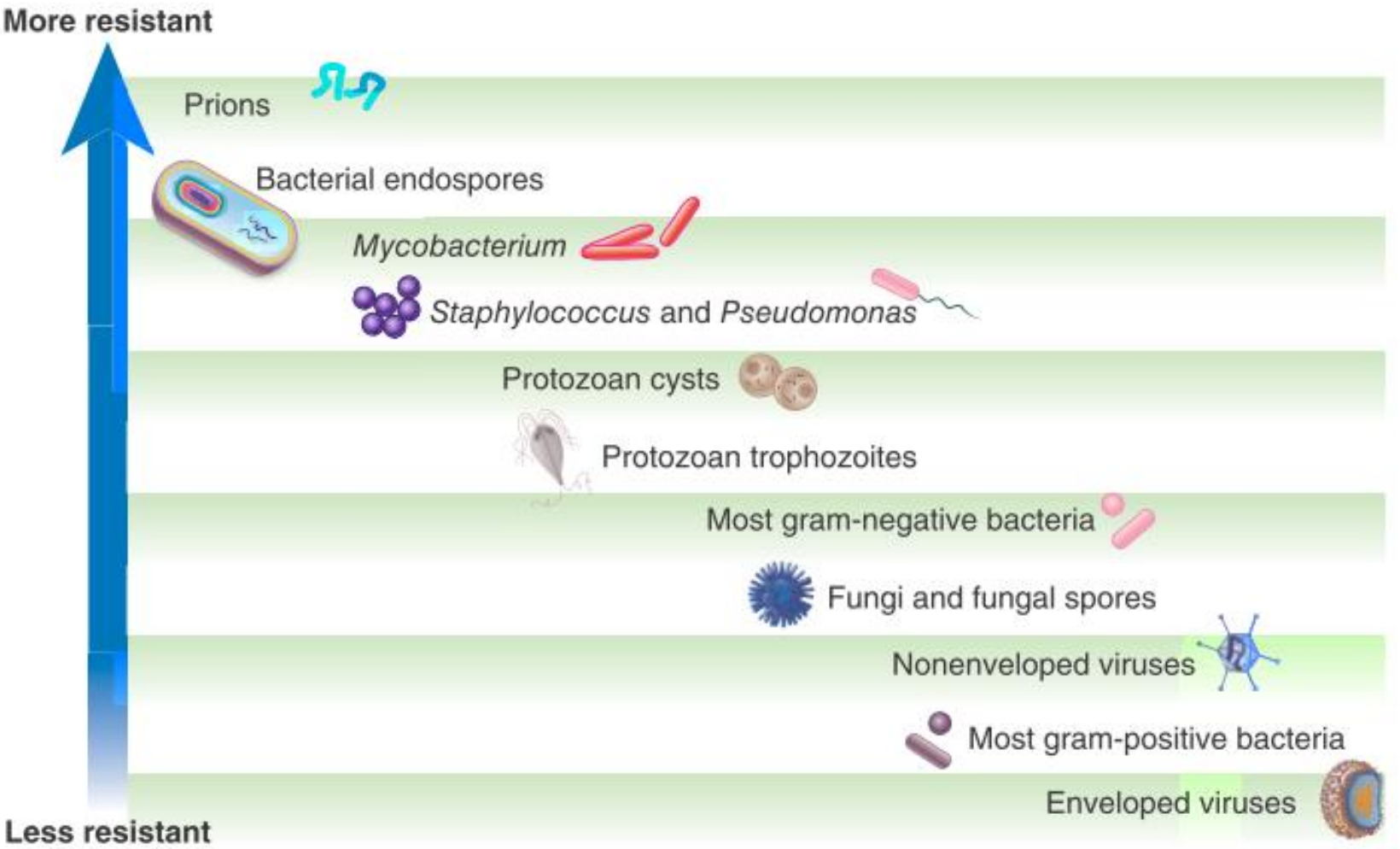
- **Sterilization:** [Latin *sterilis*, unable to produce offspring or barren] is the process by which all living cells, spores and acellular entities (e.g., viruses, viroids, and prions) are either destroyed or removed from an object or habitat. A sterile object is totally free of viable microorganisms, spores, and other infectious agents. When sterilization is achieved by a chemical agent, the chemical is called a sterilant.

- **Disinfection** is the killing, inhibition, or removal of microorganisms that may cause disease. The primary goal is to destroy potential pathogens, but disinfection also substantially reduces the total microbial population. Disinfectants are chemical used to carry out disinfection and are normally used only on inanimate objects. A disinfectant does not necessarily sterilize an object because viable spores and a few microorganisms may remain. Sanitization is closely related to disinfection.

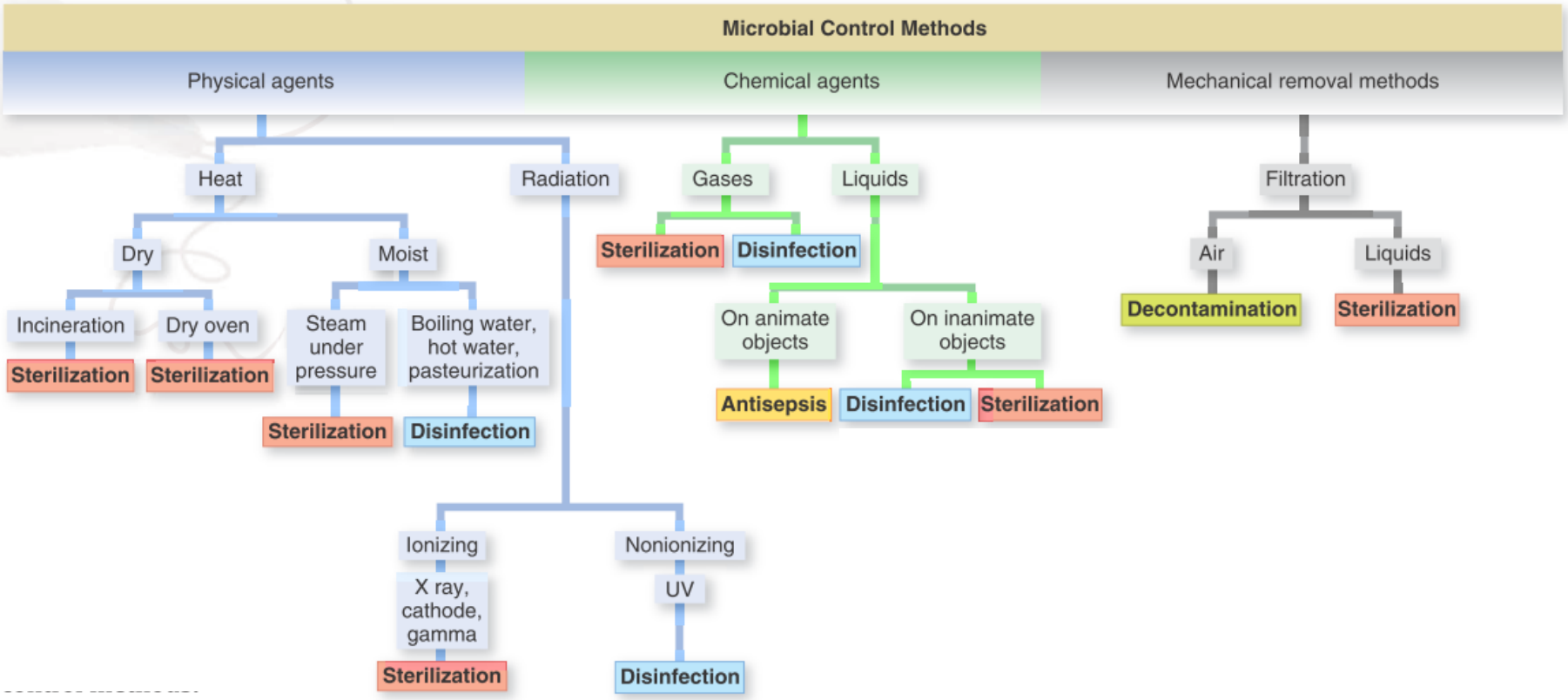
- In sanitization, the microbial population is reduced to levels that are considered safe by public health standards. The inanimate object is usually cleaned as well as partially disinfected. For example, sanitizers are used to clean eating utensils in restaurants.

- Antisepsis [Greek anti, against, and sepsis, putrefaction] is the prevention of infection or sepsis and is accomplished with antiseptics. These are chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population.

# Relative resistance of different microbial types to microbial control agents



# Microbial control methods



## Table 9.3 Actions of Various Physical and Chemical Agents upon the Cell

| Cellular Target | Effects of Agents | Examples of Agents Used |
|---|---|---|
| **Cell wall** | Chemical agents can damage the cell wall by<br>• blocking its synthesis, or<br>• digesting the cell wall. | Chemicals<br>Detergents<br>Alcohol |
| **Cytoplasmic membrane** | Agents physically bind to lipid layer of the cytoplasmic membrane, opening up the cytoplasmic membrane and allowing injurious chemicals to enter the cell and important ions to exit the cell. | Detergents |
| **Cellular synthesis** | Agents can interrupt the synthesis of proteins via the ribosomes, inhibiting proteins needed for growth and metabolism and preventing multiplication.<br>Agents can change genetic codes (mutation). | Formaldehyde<br>Radiation<br>Ethylene oxide |
| **Proteins** | Some agents are capable of denaturing proteins (breaking of protein bonds, which results in breakdown of the protein structure).<br>Agents may attach to the active site of a protein, preventing it from interacting with its chemical substrate. | Moist heat<br>Alcohol<br>Phenolics |

# Dry heat for control of microbes

- Aseptic technique in the laboratory typically involves some dry-heat sterilization protocols using direct application of high heat, such as sterilizing inoculating loops.

- **Incineration** at very high temperatures destroys all microorganisms. Dry heat can also be applied for relatively long periods of time (at least 2 hours) at temperatures up to 170 °C by using a dry-heat sterilizer, such as an oven. **Microorganisms lose viability at very high temperatures because most macromolecules lose structure and function, a process called denaturation**.

- However, **Moist heat has better penetrating power** than dry heat and, at a given temperature, produces a faster reduction in the number of living organisms.

# Dry heat for control of microbes

- The effect of dry heat on microorganisms is equivalent to that of baking. The heat changes **microbial proteins by oxidation reactions and creates an arid internal environment**, thereby burning microorganisms slowly. It is essential that organic matter such as oil or grease films be removed from the materials, because such substances insulate against dry heat.

- In the years before the development of the autoclave, liquids and other objects were sterilized by exposure to **free-flowing steam at 100°C for 30 minutes** on each of three successive days, with incubation periods at room temperature between the steaming. The method was called **fractional sterilization** because a fraction of the sterilization was accomplished on each day. It was also called **tyndallization after its developer, John Tyndall**.





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# Moist heat for control of microbes

- Moist heat readily kills viruses, bacteria, and fungi. Moist heat is thought to kill by degrading nucleic acids and by denaturing enzymes and other essential proteins. It may also disrupt cell membranes.

- The autoclave is a sealed heating device that uses steam under pressure to kill microorganisms. The autoclave uses steam under 1.1 kilograms/square centimeter ($kg/cm^2$) [15 pounds/square inch ($lb/in^2$)] pressure, which yields a temperature of 121°C.

- At this temperature saturated steam destroys all vegetative cells and endospores in a small volume of liquid within 10 to 12 minutes. Treatment is continued for at least 15 minutes to provide a margin of safety.

- Moist heat coagulates microbial proteins (including protein enzymes), inactivating them irreversibly. In the dry state, protein structures are more stable; therefore, the **temperature of dry heat must be raised much higher and maintained longer than that of moist heat**.





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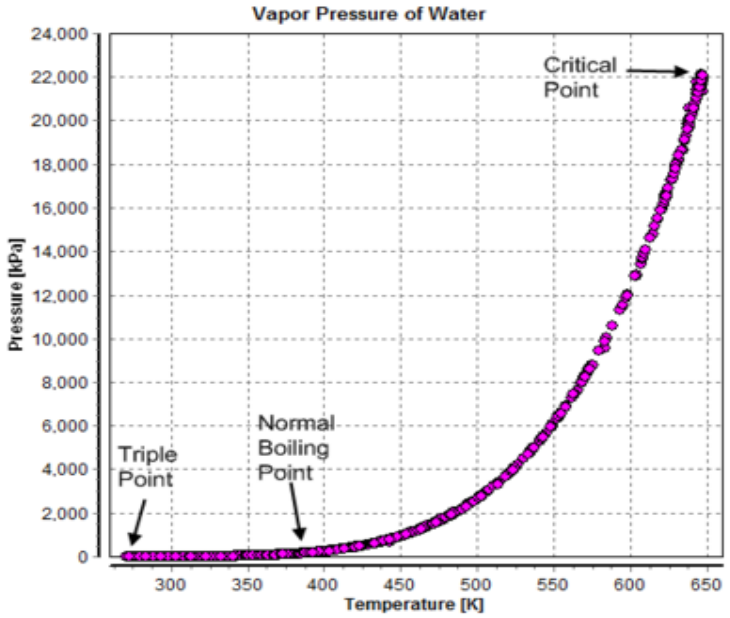
# Why is it called an autoclave?

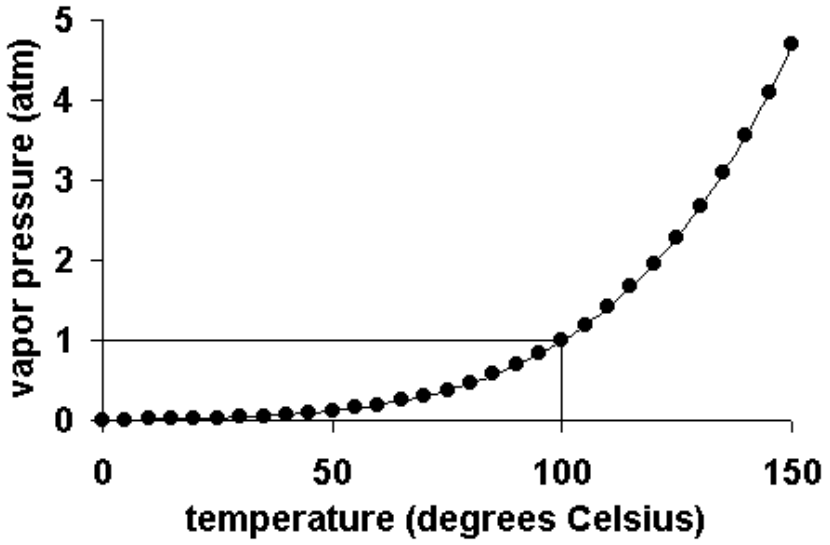
- It describes a device that automatically locks shut when the pressure rises (to avoid steam spraying out if you open it by accident). The word is French, and comes from the Greek "auto" for automatic and the Latin "clvis," for key (as in lock and key).

- It was invented by Charles Chamberland in 1884. Autoclaves sterilize or disinfect through physical means by using pressure, temperature and steam. They are often referred to as steam sterilization machines.

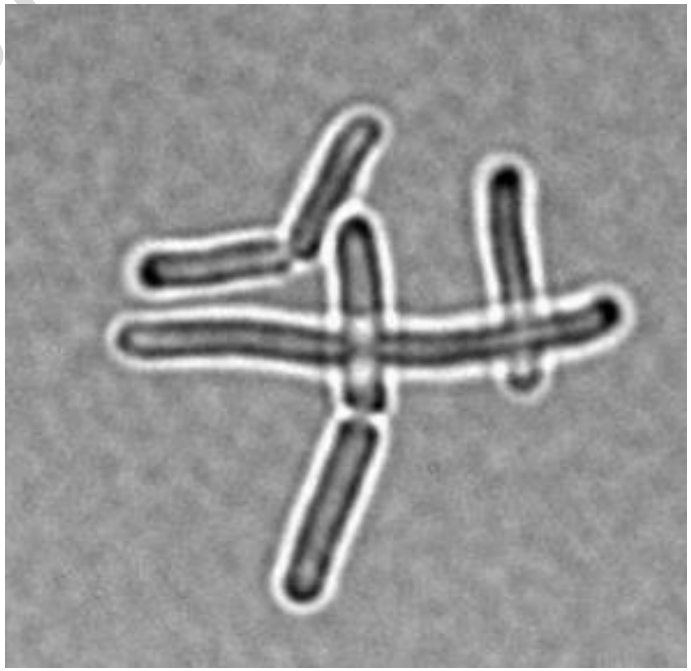
# Why is an autoclave such an effective sterilizer?

- An autoclave is a large pressure cooker; it operates by using steam under pressure as the sterilizing agent. High pressures enable steam to reach high temperatures, thus increasing its heat content and killing power. Most of the heating power of steam comes from its latent heat of vaporization. This is the amount of heat required to convert boiling water to steam. With increase in temperature of water the vapor pressure also increases.



- A biological indicator is often autoclaved along with other material. This indicator commonly consists of a culture tube containing a sterile ampule of medium and a paper strip covered with spores of *Geobacillus stearothermophilus*. After autoclaving, the ampule is aseptically broken and the culture incubated for several days. If the test bacterium does not grow in the medium, the sterilization run has been successful.



# Pasteurization

- Pasteurization uses precisely controlled heat to reduce the number of microorganisms found in milk and other heat sensitive liquids.

- The process, named for Louis Pasteur, was first used for controlling the spoilage of wine. Pasteurization does not kill all organisms and is therefore not a method of sterilization. Pasteurization does, however, reduce the microbial load, the number of viable microorganisms in a sample. In addition, by decreasing the overall microbial load, pasteurization retards the growth of spoilage organisms, increasing the shelf life of perishable liquids.
- One method for milk pasteurization, called the holding (or batch) method, involves heating at 63°C for 30 minutes. Two other methods are the flash pasteurization method at 71.6°C for 15 seconds and the ultra high temperature (UHT) method at 140°C for 3 seconds. The UHT method is the only method that sterilizes the liquid.

# Determination of heat sensitivity of an organism

- The characterization of heat sensitivity of an organism is to measure the thermal death time. The lowest temperature required to sterilize a standardized pure culture of bacteria within a given time can be called the **thermal death point** of that species. Conversely, the time required to sterilize the culture at a stated temperature can be established as the **thermal death time**.
- To determine the thermal death time, samples of a cell suspension are heated for different times, mixed with culture medium, and incubated. If all the cells have been killed, no growth is observed in the incubated samples.
- The thermal death time depends on the size of the population tested; a longer time is required to kill all cells in a large population than in a small one. When the number of cells is standardized, it is possible to compare the heat sensitivities of different organisms by comparing their thermal death times at a given temperature.

# Endospores and Heat Sterilization

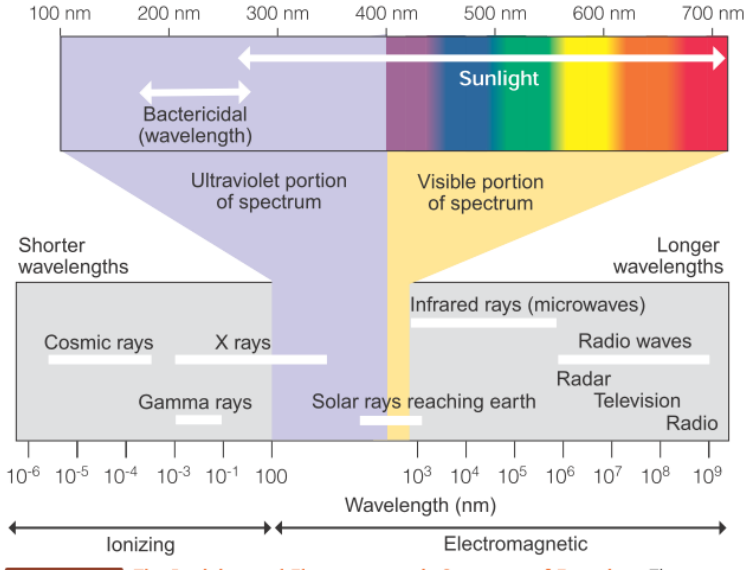
Endospores can survive heat that would rapidly kill vegetative cells of the same species. A major factor in heat resistance is the amount and state of water within the endospore. During endospore formation, the protoplasm is reduced to a minimum volume as a result of the accumulation of calcium ($Ca^{2+}$)–dipicolinic acid complexes and small acid-soluble spore proteins (SASPs). This mixture forms a cytoplasmic gel, and a thick cortex then forms around the developing endospore. Contraction of the cortex results in a shrunken, dehydrated cell containing only 10–30% of the water of a vegetative cell.

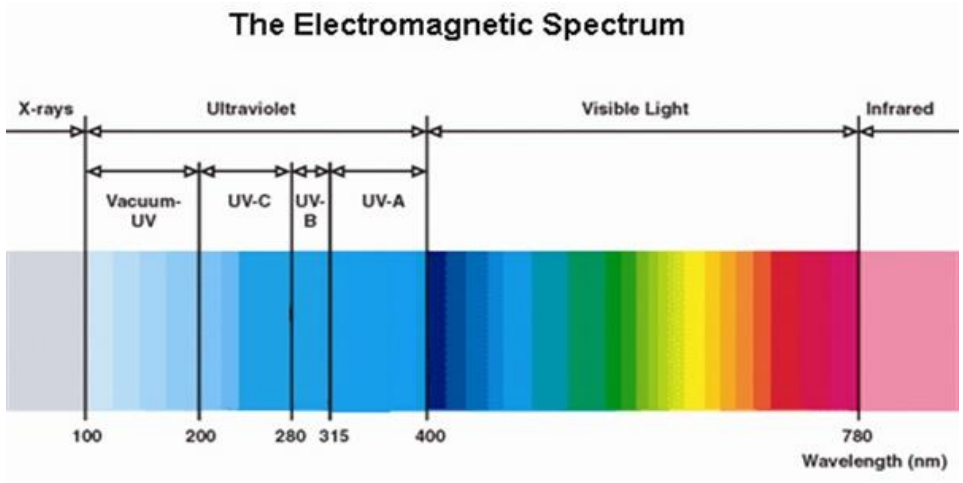
Heat sterilization of dry objects such as endospores always requires higher temperatures and longer heat application times than sterilization of wet objects.

# Ultraviolet Radiation

- ✓ Ultraviolet radiation between 220 and 300 nm in wavelength has enough energy to cause modifications or actual breaks in DNA, sometimes leading to disruption of DNA and death of the exposed organism.

- ✓ This UV light is useful for disinfecting surfaces, air, and materials such as water that do not absorb the UV waves. UV radiation, cannot penetrate solid, opaque, or light-absorbing surfaces, limiting its use to disinfection of exposed surfaces.



# Low temperature

- Refrigeration greatly slows microbial growth and reproduction, but does not halt it completely. Freezing items at -20°C or lower stops microbial growth because of the low temperature and the absence of liquid water. Some microorganisms will be killed by ice crystal disruption of cell membranes, but freezing does not destroy all contaminating microbes.
- Freezing (-30°C or -70°C) is a very good method for long-term storage of microbial samples under optimized conditions.
- This effect may be because of stiffening of the lipids of the membrane at lower temperature, leading to decreased efficiency of transport proteins embedded in the membrane. Therefore, an organism unable to supply the growth rate-limiting nutrient due to loss of affinity for that substrate.





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# Ionizing Radiation

- Ionizing radiation is electromagnetic radiation of sufficient energy to produce ions and other reactive molecular species from molecules with which the radiation particles collide. Ionizing radiation generates electrons, hydroxyl radicals ($OH^{\cdot}$) and hydride radicals ($H^{\cdot}$). Each of these highly reactive molecules is capable of altering and disrupting macromolecules such as DNA, lipids, and protein. The ionization and subsequent degradation of these biologically important molecules leads to the death of irradiated cells.

- The sources of ionizing radiation include cathode ray tubes that generate electron beams, X-ray machines, and radioactive nuclides. These sources produce electrons (e-), X-rays, or γ-rays, respectively, all of which have sufficient energy to efficiently kill microorganisms. In addition, X-rays and γ-rays penetrate solids and liquids, making them ideal for treatment of bulk items.

# Desiccation

- Drying, also known as desiccation or dehydration, is a method that has been used for millennia to preserve foods.

- It works because all cells, including microbes, require water for their metabolism and survival. Although drying controls microbial growth, it might not kill all microbes or their endospores, which may start to regrow when conditions are more favorable and water content is restored.

- Susceptibility to dessication varies widely:

***Neisseria gonnorrhea*: Only survives about one hour.**
***Mycobacterium tuberculosis*: May survive several months.**
**Viruses are fairly resistant to desiccation**





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# High pressure

- Exposure to high pressure kills many microbes. In the food industry, high-pressure processing is used to kill bacteria, yeast, molds, parasites, and viruses in foods while maintaining food quality and extending shelf life.

- High pressure affects cellular physiology and biochemistry in many ways. In general, pressure decreases the ability of the subunits of multi-subunit proteins to interact. Protein synthesis, DNA synthesis, and nutrient transport are sensitive to high pressure.

- Pascalization, also known as High Pressure Processing or Bridgmanization, is a method of preserving and sterilizing food by placing the product under extremely high pressure in order to inactivate certain microorganisms and enzymes.

# Osmotic Pressure

- Preservation by salting is based upon the principle of osmotic pressure. When food is salted (usually sodium chloride), water diffuses out of microorganisms toward the higher salt concentration and lower water concentration in the surrounding environment.
- This flow of water, called osmosis, leaves the microorganisms dehydrated, and they die. The same phenomenon occurs in highly sugared foods (usually sucrose) such as syrups, jams, and jellies. The use of **high concentrations of salts and sugars** in foods is used to increase the osmotic pressure and create a hypertonic environment.

- Plasmolysis: As water leaves the cell, plasma membrane shrinks away from cell wall. Cell may not die, but usually stops growing.





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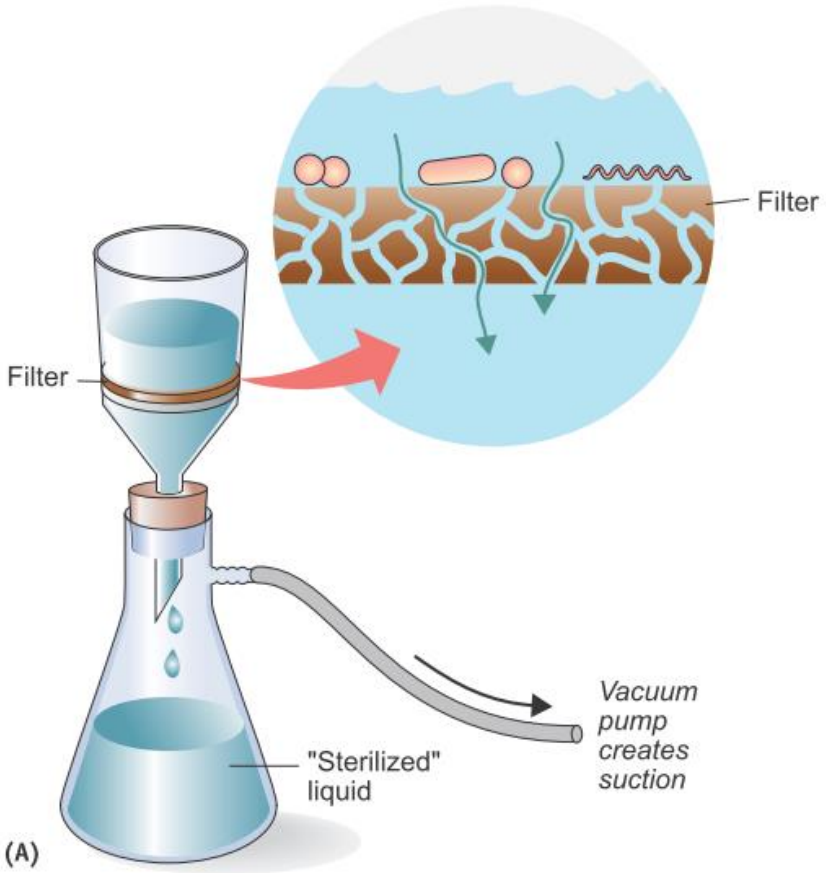
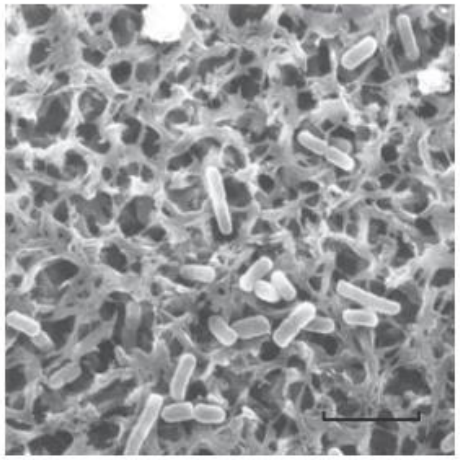
# Filter Sterilization

- Heat-sensitive liquids must be decontaminated and even sterilized without exposure to denaturing heat. The liquid or gas is passed through a filter, a device with pores too small for the passage of microorganisms, but large enough to allow the passage of the liquid or gas.
- The selection of filters for sterilization must account for the size range of the contaminants to be excluded. Although a wide variety of pore sizes are available, membranes with pores about 0.2 µm in diameter are used to remove most vegetative cells.
- In laminar flow biological safety cabinets, high-efficiency particulate air (HEPA) filters (a type of depth filter) is used to remove 99.97% of 0.3 µm particles. Laminar flow **biological safety cabinets** or hoods force air through HEPA filters, then project a vertical curtain of sterile air across the cabinet opening.

# Membrane Filters

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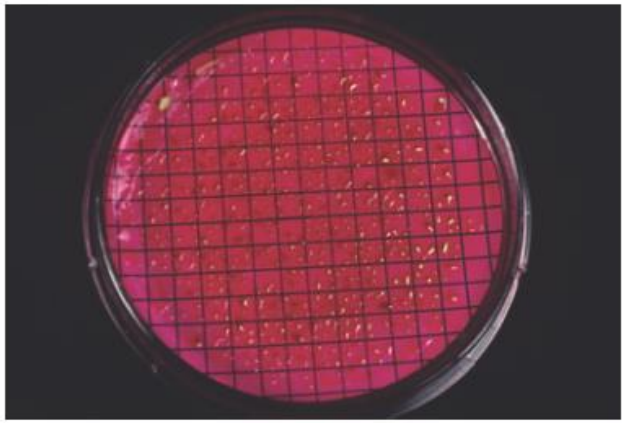
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# Chemical Antimicrobial Control

- An antimicrobial agent is a natural or synthetic chemical that kills or inhibits the growth of microorganisms. They are called bacteriocidal, fungicidal, and viricidal agents because they kill bacteria, fungi, and viruses, respectively.

- Agents that do not kill but only inhibit growth are called –static agents. These include bacteriostatic, fungistatic, and viristatic compounds.

- Antibacterial agents can be classified as bacteriostatic, bacteriocidal, and bacteriolytic by observing their effects on bacterial cultures.





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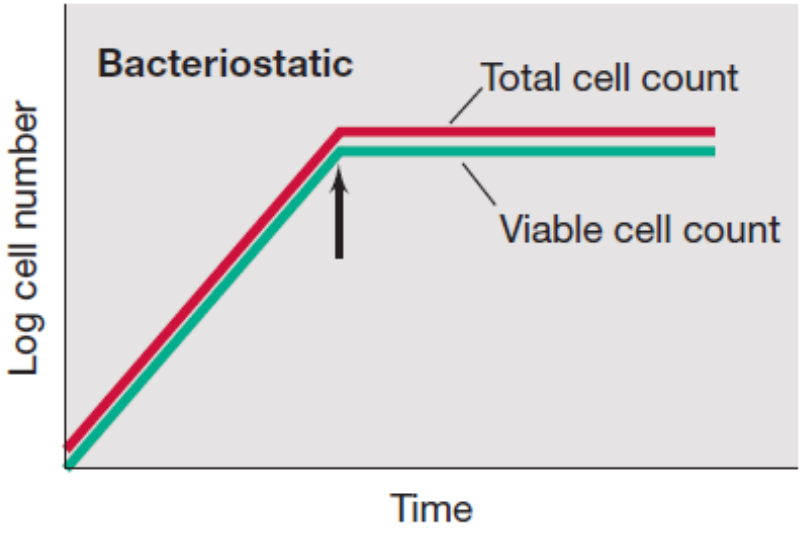
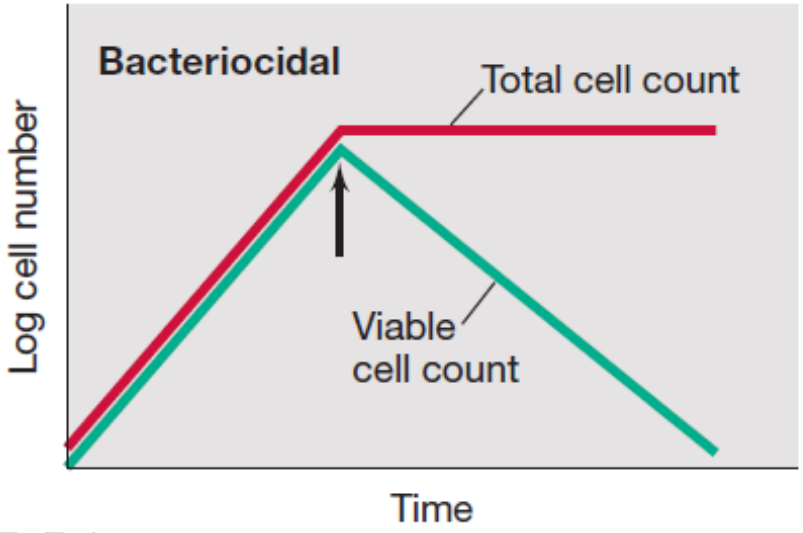
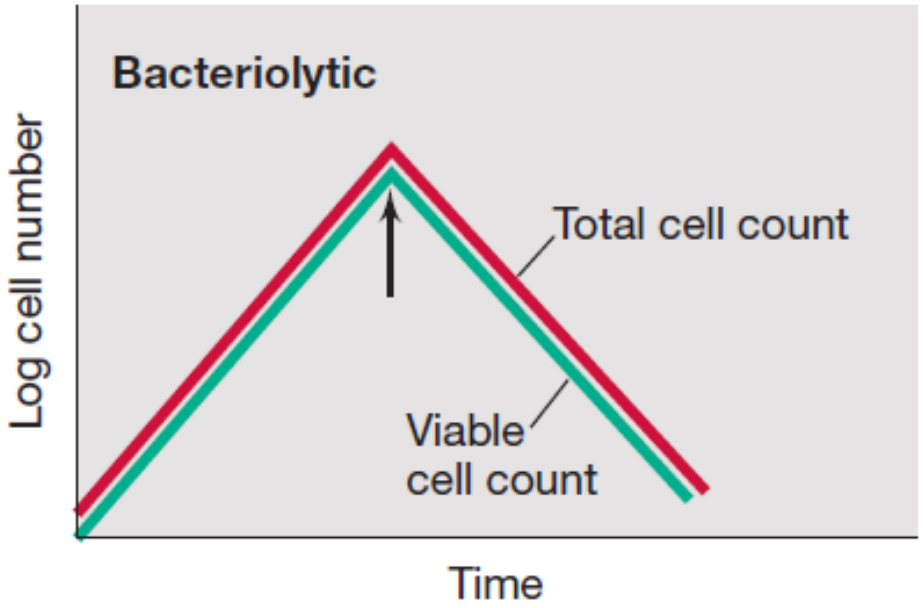
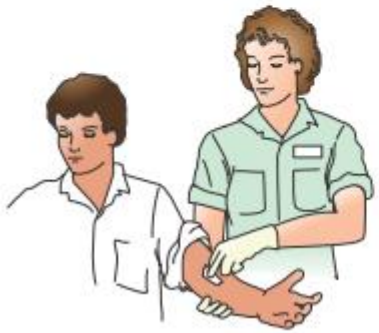


- The process of destroying pathogens is called **disinfection** and the object is said to be **disinfected**.

- If the object treated is lifeless, the chemical agent used is called a **disinfectant**. However, if the object treated is living, such as a tissue of the human body, the chemical agent used is an antiseptic.



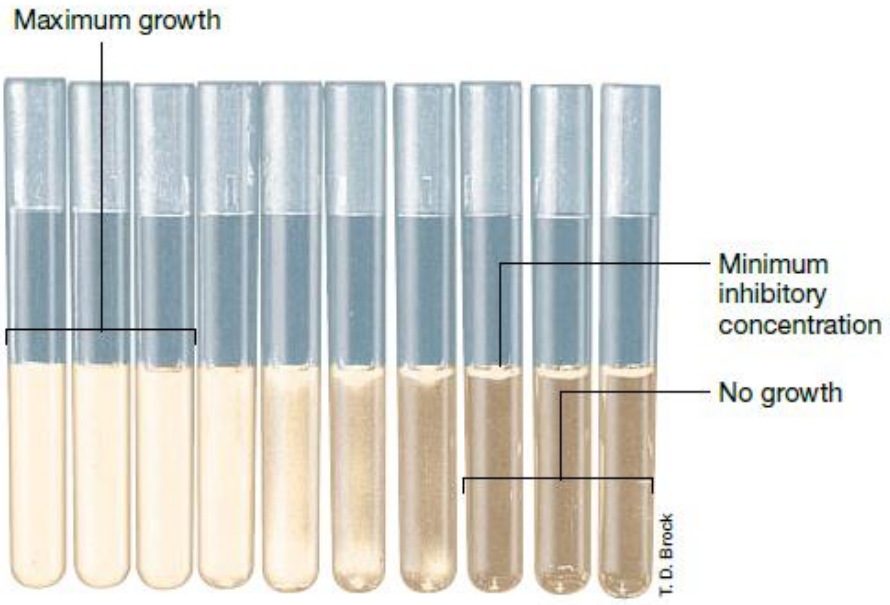
**Antiseptics**

**Disinfectants**

# Measuring Antimicrobial Activity

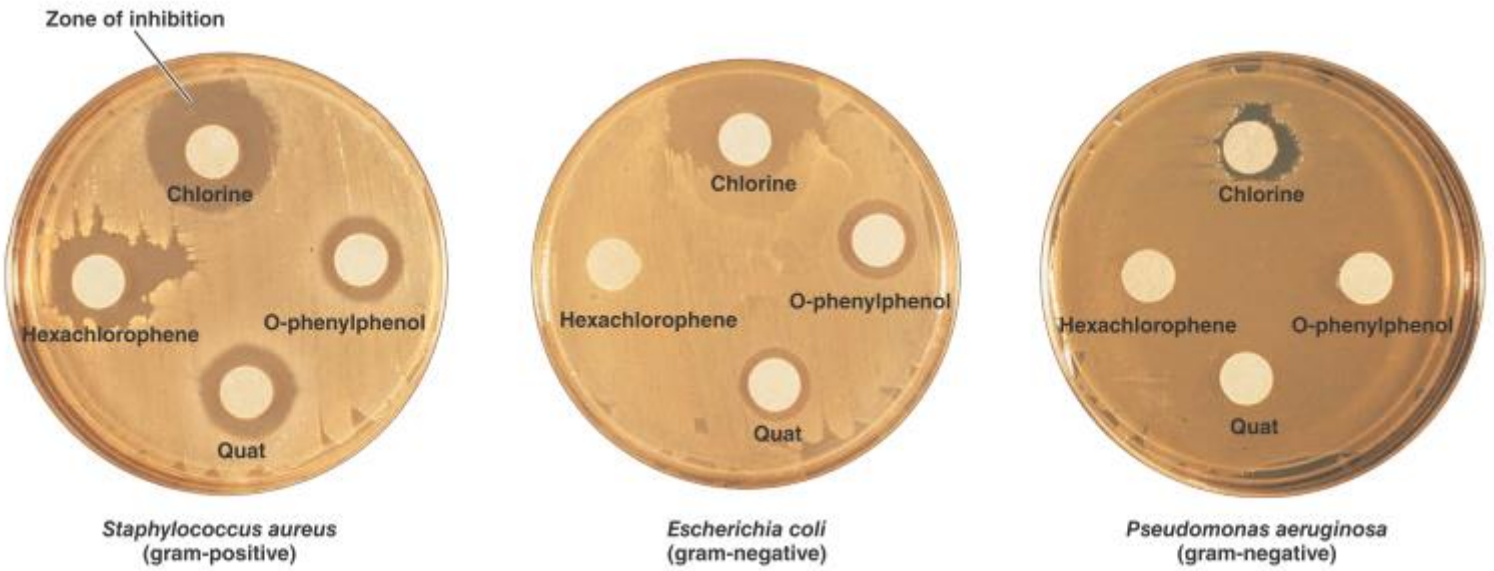
Antimicrobial activity is measured by determining the smallest amount of agent needed to inhibit the growth of a test organism, a value called the minimum inhibitory concentration (MIC).

The MIC is the lowest concentration of agent that completely inhibits the growth of the test organism. This is called the tube dilution technique.



# The Disk-Diffusion Method

- The disk-diffusion method is used in teaching laboratories to evaluate the efficacy of a chemical agent. A disk of filter paper is soaked with a chemical and placed on an agar plate that has been previously inoculated and incubated with the test organism. After incubation, if the chemical is effective, a clear zone representing inhibition of growth can be seen around the disk.



| Agent | Target Microbes | Form(s) | Mode of Action | Indications for Use | Limitations |
|---|---|---|---|---|---|
| **Halogens: chlorine**   | Can kill endospores (slowly); all other microbes | Liquid/gaseous chlorine ($Cl_2$), hypochlorites (OCl), chloramines ($NH_2Cl$) | In solution, these compounds combine with water and release hypochlorous acid (HOCl); denature enzymes permanently and suspend metabolic reactions | Chlorine kills bacteria, endospores, fungi, and viruses; gaseous/liquid chlorine: used to disinfect drinking water, sewage and waste water; hypochlorites: used in health care to treat wounds, disinfect bedding and instruments, sanitize food equipment and in restaurants, pools, and spas; chloramines: alternative to pure chlorine in treating drinking water; also used to treat wounds and skin surfaces | Less effective if exposed to light, alkaline pH, and excess organic matter |
| **Halogens: iodine** | Can kill endospores (slowly); all other microbes | Free iodine in solution ($I_2$)<br>Iodophors (complexes of iodine and alcohol) | Penetrates cells of microorganisms where it interferes with a variety of metabolic functions; interferes with the hydrogen and disulfide bonding of proteins | 2% iodine, 2.4% sodium iodide (aqueous iodine) used as a topical antiseptic<br>5% iodine, 10% potassium iodide used as a disinfectant for plastic and rubber instruments, cutting blades, etc.<br>Iodophor products contain 2% to 10% of available iodine, which is released slowly; used to prepare skin for surgery, in surgical scrubs, to treat burns, and as a disinfectant | Can be extremely irritating to the skin and is toxic when absorbed |
| **Hydrogen peroxide ($H_2O_2$)**   | Kills endospores and all other microbes | Colorless, caustic liquid<br>Decomposes in the presence of light metals or catalase into water, and oxygen gas | Oxygen forms free radicals (−OH), which are highly toxic and reactive to cells | As an antiseptic, 3% hydrogen peroxide used for skin and wound cleansing, mouth washing, bedsore care<br>Used to treat infections caused by anaerobic bacteria<br>35% hydrogen peroxide used in low temperature sterilizing cabinets for delicate instruments | Sporicidal only in high concentrations |

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| **Aldehydes** | Kill endospores and all other microbes | Organic substances bearing a –CHO functional group on the terminal carbon | Glutaraldehyde can irreversibly disrupt the activity of enzymes and other proteins within the cell<br>Formaldehyde is a sharp irritating gas that readily dissolves in water to form an aqueous solution called formalin; attaches to nucleic acids and functional groups of amino acids | Glutaraldehyde kills rapidly and is broad-spectrum; used to sterilize respiratory equipment, scopes, kidney dialysis machines, dental instruments<br>Formaldehyde kills more slowly than glutaraldehyde; used to disinfect surgical instruments | Glutaraldehyde is somewhat unstable, especially with increased pH and temperature<br>Formaldehyde is extremely toxic and is irritating to skin and mucous membranes |
| **Gaseous sterilants/ disinfectants** | Ethylene oxide kills endospores; other gases less effective | Ethylene oxide is a colorless substance that exists as a gas at room temperature | Ethylene oxide reacts vigorously with functional groups of DNA and proteins, blocking both DNA replication and enzymatic actions<br>Chlorine dioxide is a strong alkylating agent | Ethylene oxide is used to disinfect plastic materials and delicate instruments; can also be used to sterilize syringes, surgical supplies, and medical devices that are prepackaged | Ethylene oxide is explosive–it must be combined with a high percentage of carbon dioxide or fluorocarbon<br>It can damage lungs, eyes, and mucous membranes if contacted directly<br>Ethylene oxide is rated as a carcinogen by the government |

| Agent | Target Microbes | Form(s) | Mode of Action | Indications for Use | Limitations |
|---|---|---|---|---|---|
| **Phenol (carbolic acid)** | Some bacteria, viruses, fungi | Derived from the distillation of coal tar<br>Phenols consist of one or more aromatic carbon rings with added functional groups | In high concentrations, they are cellular poisons, disrupting cell walls and membranes, proteins<br>In lower concentrations, they inactivate certain critical enzyme systems | Phenol remains one standard against which other (less toxic) phenolic disinfectants are rated; the phenol coefficient quantitatively compares a chemical's antimicrobial properties to those of phenol<br>Phenol is now used only in certain limited cases, such as in drains, cesspools, and animal quarters | Toxicity of many phenolics makes them dangerous to use as antiseptics |

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| **Chlorhexidine** | Most bacteria, viruses, fungi | Complex organic base containing chlorine and two phenolic rings | Targets both bacterial membranes, where selective permeability is lost, and proteins, resulting in denaturation | Mildness, low toxicity and rapid action make chlorhexidine a popular choice of agents<br>Used in hand scrubs, prepping skin for surgery, as an obstetric antiseptic, as a mucous membrane irrigant, etc. | Effects on viruses and fungi are variable |
| **Alcohol**  | Most bacteria, viruses, fungi | Colorless hydrocarbons with one or more –OH functional groups<br>Ethyl and isopropyl alcohol are suitable for antimicrobial control | Concentrations of 50% and higher dissolve membrane lipids, disrupt cell surface tension, and compromise membrane integrity | Germicidal, nonirritating, and inexpensive<br>Routinely used as skin degerming agents (70% to 95% solutions) | Rate of evaporation decreases effectiveness<br>Inhalation of vapors can affect the nervous system |
| **Detergents** | Some bacteria, viruses, fungi | Polar molecules that act as surfactants<br>Anionic detergents have limited microbial power<br>Cationic detergents, such as quaternary ammonium compounds ("quats"), are much more effective antimicrobials | Positively charged end of the molecule binds well with the predominantly negatively charged bacterial surface proteins<br>Long, uncharged hydrocarbon chain allows the detergent to disrupt the cytoplasmic membrane<br>Cytoplasmic membrane loses selective permeability, causing cell death | Effective against viruses, algae, fungi, and gram-positive bacteria<br>Rated only for low-level disinfection in the clinical setting<br>Used to clean restaurant utensils, dairy equipment, equipment surfaces, restrooms | Ineffective against tuberculosis bacterium, hepatitis virus, *Pseudomonas*, and endospores<br>Activity is greatly reduced in presence of organic matter<br>Detergents function best in alkaline solutions |

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| **Heavy metal compounds**  | Some bacteria, viruses, fungi | Heavy metal germicides contain either an inorganic or an organic metallic salt; may come in tinctures, soaps, ointment, or aqueous solution | Mercury, silver, and other metals exert microbial effects by binding onto functional groups of proteins and inactivating them | Organic mercury tinctures are fairly effective antiseptics<br>Organic mercurials serve as preservatives in cosmetics, ophthalmic solutions, and other substances<br>Silver nitrate solutions are used for topical germicides and ointments | Microbes can develop resistance to metals<br>Not effective against endospores<br>Can be toxic if inhaled, ingested, or absorbed<br>May cause allergic reactions in susceptible individuals |