Lecture 6. Staining techniques - principle and types of stains - staining techniques- simple, negative, differential and structural staining methods

Although living microorganisms can be directly examined with the light microscope, they often must be fixed and **stained** to increase **visibility, highlight specific morphological features, and preserve them for future study**.

. FIXATION

Fixation is the process by which the internal and external structures of cells and microorganisms are preserved and fixed in position. It inactivates enzymes that might disrupt cell morphology and toughens cell structures so that they do not change during staining and observation. A microorganism usually is killed and attached firmly to the microscope slide during fixation. There are two fundamentally different types of fixation.

1. **Heat fixation**

   Bacterial smear will be gently showed in flame. This adequately preserves overall morphology but not structures within cells.

2. **Chemical fixation**

   Chemical fixatives are used that will penetrate cells and react with cellular components, usually proteins and lipids, to render them inactive, insoluble, and immobile. Common fixative mixtures contain such components as ethanol, acetic acid, mercuric chloride, formaldehyde, and glutaraldehyde.

DYES

The many types of dyes used to stain microorganisms. They may be anyone of the following types:

1. They may be **chromophore groups** (groups with conjugated double bonds) that give the dye its color.
2. **Ionizable dyes** (bind with cells by ionic, covalent, or hydrophobic bonding). For example, a positively charged dye binds to negatively charged structures on the cell. Ionizable dyes may be divided into two general classes based on the nature of their charged group.
   a) **Basic dyes** - Basic dyes are cationic. Ex: Methylene blue, basic fuchsin, crystal violet, safranin, malachite green. They have positively charged groups (usually some form of pentavalent nitrogen) and are generally available as chloride salts. Basic dyes bind to negatively charged molecules like nucleic acids and many proteins. Because the surfaces of bacterial cells also are negatively charged, basic dyes are most often used in bacteriology.

b) **Acid dyes** – Acid dyes are anionic. Ex: Eosin, rose bengal, and acid fuchsin. They possess negatively charged groups such as carboxyls (—COOH) and phenolic hydroxyls (—OH). Acid dyes, because of their negative charge, bind to positively charged cell structures.

STANING REACTION

The process of staining may involve **ion-exchange reactions** between the stain and active sites at the surface of or within the cell. For example, the colored ions of the dye may replace other ions on cellular components. Certain chemical groupings of cell proteins or nucleic acids may be involved in salt formation with positively charged ions such as Na^+ or K^+. Thus we might view these peripheral areas of the cell as carrying a negative charge in combination with positively charged ions; for example,

$$(\text{Bacterial cell}^-)\ (Na^+)$$

In a basic dye like methylene blue, the colored ion is positively charged (a cation), and if we represent this ion by the symbol MB, the dye, which is actually methylene blue chloride, may be represented as

$$(MB^+)\ (Cl^-)$$

The ionic exchange which takes place during staining can be represented by the following equation, in which the MB^+ cation replaces the Na^+ cation in the cell:

$$(\text{Bacterial cell}^-)\ (Na^+) + (MB^+)\ (Cl^-) \rightarrow (\text{Bacterial cell}^-)\ (MB^+) + (Na^+Cl^-)$$

. TYPES OF STAINING

SIMPLE STAINING

Microorganisms often can be stained very satisfactorily by simple staining**,** in which a single staining agent is used. Simple staining value lies in its simplicity and ease of use. One covers the fixed smear with stain for the proper length of time, washes the excess stain off with water, and blots the slide dry. In simple staining only one stain is used. Basic dyes like crystal violet, methylene blue, and carbolfuchsin are frequently used to determine the size, shape, and arrangement of bacteria. It is of two types:

1. **Positive staining / Direct staining**

   In which basic dyes are used. So the cells are absorbing the colour of the dye. Here cells appear coloured in bright background.

2. **Negative staining / Indirect staining**

   In which acidic dyes are used. The cells will not take up the colour and instead background is stained. Here cells appear transparent against the stained or dark background.

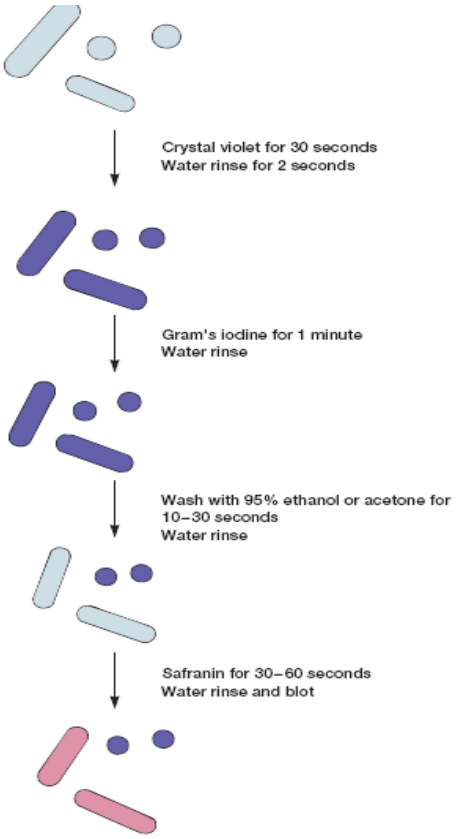
DIFFERENTIAL STAINING

In differential staining more than one stain is used. The basic dyes are used. Differential staining procedures divide bacteria into separate groups based on staining properties. Differential staining includes,

1. Gram staining
2. Capsule staining and
3. Flagella staining
4. Endospore staining

. GRAM STAINING

Gram stain was developed in 1884 by the Danish physician Christian Gram, is the most widely employed staining method in bacteriology. It is a differential staining procedure because it divides bacteria into two classes - gram negative and gram positive.



In the first step of the Gram-staining procedure, the smear is stained with the basic dye crystal violet, the primary stain. It is followed by treatment with an iodine solution functioning as a **mordant.** That is, the iodine increases the interaction between the cell and the dye so that the cell is stained more strongly. The smear is next decolorized by washing with ethanol or acetone. This step generates the differential aspect of the Gram stain; gram-positive bacteria retain the crystal violet, whereas gram-negative bacteria lose their crystal violet and become colorless. Finally, the smear is counterstained with a simple, basic dye different in color from crystal violet. Safranin, the most common counterstain, colors gram-negative bacteria pink to red and leaves gram-positive bacteria dark purple.

The more possible explanation for the Gram reaction of a bacterial cell is associated with the structure and composition of the cell wall. The cell wall of Gram negative bacteria are generally thinner than those of Gram positive bacteria. Gram negatives contain a higher percentage of lipids than Gram positives. During staining of the Gram negative bacteria, the alcohol treatment extracts the lipids, which results in increased porosity or permeability of the cell wall. Thus the crystal violet-iodine (CV-I) complex can be extracted and the Gram negative organism is decolourized. These cells subsequently take on the colour of the Safranin, counter strain. The cell walls of Gram positive bacteria, because of their different composition (less lipids) become dehydrated during alcohol addition. The pore size decreases, permeability is reduced and the CV-I complex cannot be extracted. Therefore these cells remain purple-violet.

. ACID-FAST STAINING

It is another important differential staining procedure. A few species, particularly those in the genus ***Mycobacterium*** do not bind simple stains readily and must be stained by a harsher treatment: heating with a mixture of basic fuchsin and phenol (the Ziehl-Neelsen method).

Once basic fuchsin has penetrated with the aid of heat and phenol, acid-fast cells are not easily decolorized by an acid-alcohol wash and hence remain red. This is due to the quite high lipid content of acid-fast cell walls; in particular, mycolic acid—a group of branched chain hydroxy lipids—appears responsible for acidfastness. Non-acid-fast bacteria are decolorized by acid-alcohol and thus are stained blue by methylene blue counterstain. This method is used to identify *Mycobacterium tuberculosis* and *M. leprae*, the pathogens responsible for tuberculosis and leprosy, respectively.

. STAINING SPECIFIC STRUCTURES

Many special staining procedures have been developed over the years to study specific bacterial structures with the light microscope.

1. NEGATIVE STAINING

One of the simplest is **negative staining,** a technique that reveals the presence of the diffuse capsules surrounding many bacteria. Bacteria are mixed with Indian ink or Nigrosin dye and spread out in a thin film on a slide. After air-drying, bacteria appear as lighter bodies in the midst of a blue-black background because ink and dye particles cannot penetrate either the bacterial cell or its capsule. The extent of the light region is determined by the size of the capsule and of the cell itself. There is little distortion of bacterial shape, and the cell can be counterstained for even greater visibility.

2. ENDOSPORE STAINING

Bacteria in the genera *Bacillus* and *Clostridium* form an exceptionally resistant structure capable of surviving for long periods in an unfavorable environment. This dormant structure is called an endospore since it develops within the cell. Endospore morphology and location vary with species and often are valuable in identification; endospores may be spherical to elliptical and either smaller or larger than the diameter of the parent bacterium. They can be observed with the phase-contrast microscope or negative staining. Endospores are not stained well by most dyes, but once stained, they strongly resist decolorization. This property is the basis of most **spore staining** methods.

3. FLAGELLA STAINING

Bacterial flagella are fine, threadlike organelles of locomotion that are so slender (about 10 to 30 nm in diameter) they can only be seen directly using the electron microscope. To observe them with the light microscope, the thickness of flagella is increased by coating them with mordants like tannic acid and potassium alum, and they are stained with

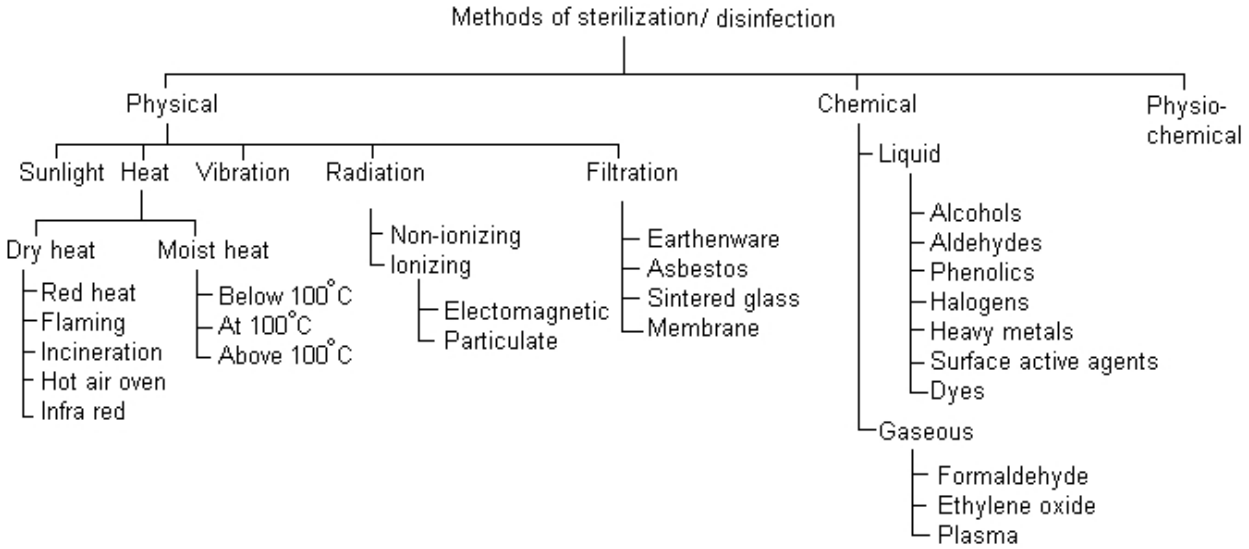
pararosaniline (Leifson method) or basic fuchsin (Gray method). Flagella staining procedures provide taxonomically valuable information about the presence and distribution pattern of flagella.

IMPORTANT STAINING TECHNIQUES AND THEIR APPLICATION

S.No	Staining technique	Application
1	Acid-fast stain	Distinguishes acid-fast bacteria such as *Mycobacterium* spp. from non-acid-fast bacteria
2	Endospore stain	Demonstrates spore structure in bacteria as well as free spores
3	Capsule stain	Demonstrates presence of capsules surrounding cells
4	Flagella stain	Demonstrates presence and arrangement of flagella

8. Sterilization – principle – physical agents and chemical methods

Sterilization [Latin *sterilis,* unable to produce offspring or barren] is the process by which all living cells, viable spores, viruses, and viroids 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 agents, usually 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. It is frequently necessary to control microorganisms on living tissue with chemical agents. **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. Because they must not destroy too much host tissue, antiseptics are generally not as toxic as disinfectants. **Cide** - A suffix can be employed to denote the type of antimicrobial agent. Substances that kill organisms often have the suffix –cide [Latin *cida,* to kill]: **Germicide** kills pathogens (and many nonpathogens) but not necessarily endospores. A disinfectant or antiseptic can be particularly effective against a specific group, in which case it may be called a **bactericide, fungicide, algicide,** or **viricide.** Other chemicals do not kill, but they do prevent growth. **Static** - If these agents are removed, growth will resume. Their names end in -static [Greek *statikos,* causing to stand or stopping]—for example, **bacteriostatic** and **fungistatic.**



. PHYSICAL AGENTS FOR MICROBIAL CONTROL

Heat and other physical agents are normally used to control microbial growth and sterilize objects, as can be seen from the continual operation of the autoclave in every microbiology laboratory. The four most frequently employed physical agents are **heat, low temperatures, filtration and radiation.**

HIGH TEMPERATURE HEATING

Fire and boiling water have been used for sterilization and disinfection since the time of the Greeks, and heating is still one of the most popular ways to destroy microorganisms. Either moist or dry heat may be applied. The heat susceptibility of the organism should be known before applying the heat treatment. For this, the thermal death time and thermal death points are essential.

a) **Thermal death time** refers the time to kill a suspension of organisms at a specific temperature.

b) **Thermal death point** refers the lowest temperature to kill a suspension in a given time.

c) **Decimal reduction time** (D time) – Time to kill 90% or 1 log unit of a population at a given temperature

Based on the above parameters, the time and temperature to kill the microorganism from an environment is derived. Practically heat can be applied either dry or moist heat for sterilization.

. MOIST HEAT

1. STEAM UNDER PRESSURE

Heat in the form of saturated steam under pressure is the most practical and dependable agent for sterilization. Steam under pressure (moist heat) provides temperature above boiling. In addition, it has the advantage of rapid heating, penetration and moisture in abundance which facilitates coagulation of proteins. Moist heat causes denaturation and coagulation of protein.

Autoclave is used for sterilization using moist heat under pressure. Autoclave is made up of double walled steel plates with air tight lids. Electrical coil submerged in water is provided to generate the steam. A pressure gauge to measure the pressure, and a safety valve for safety, are also attached in the lid. If the steam pressure inside the closed vessel is increased to 15 lb/sq. inch, the temperature will raise to 121.6°C. Keeping this condition for 15 – 20 min. will kill all the vegetative and spore structures of microbes.

2. BOILING

Vegetative forms are killed in minutes. However, it is unreliable for killing spores.

3. STEAM AT ATMOSPHERIC PRESSURE

Steam has latent heat and at 100º C has 540 calories. Latent heat is released when steam condenses on a cold surface causing proteins to coagulate.

4. PASTEURIZATION

It refers to removal of undesired microorganisms by heating at particular temperature without affecting the beneficial microorganisms, odour and taste. It is a rapid method. The pasteurization of milk is done to eliminate the pathogenic microorganisms which cause diseases like tuberculosis, brucellosis, Q fever, typhoid etc. In broad, the pasteurization is done to kill *Salmonella* and *E. coli* like organisms. There are two types of pasteurization. They are as follows:

a) **Flash pasteurization**: holding the substance at 71°C for 15 seconds and rapid cooling referred as flash pasteurization. Also referred as High Temperature Short Time (HTST) method

b) **Bulk pasteurization**: holding the substance at 63-65°C for 30 min and slow cooling referred as bulk pasteurization. Also referred as Low Temperature Holding (LTH) method

5.TYNDALLIZATION

Refers to intermittent or fractional sterilization. The subsequent cooling and heating by steam at 100^{o}C for 3 days will remove the germs and their spores. Ex. Soil which contains diversified microbes with spores. On the first day all the vegetative cells will be killed and by the subsequent days germinated spores will be killed. The normal sterilization by autoclave will eliminate the only vegetative cells and not spores. The apparatus used for tyndallization is the steam Arnold, in which free flowing steam is used.

Tyndallization > sterilization (by autoclave**) > pasteurization** is the order of degree of strength in terms of removal of microorganisms.

DRY HEAT

1. HOT AIR STERILIZATION

Causes oxidation of cells. Penetrating ability is less than moist heat. By using electrical coils, heat is generated and used for sterilization. Normally high temperature and long time are required for complete sterilization using this method. 160-180°C for 2- 3 hours are required for complete sterilization.

Hot air oven is used in this case. A double walled (inner and outer walls) instrument protected with asbestos sheets to avoid heat loss. Thermostat is present to control the temperature and timer is also present to turn off automatically after particular time. The glasswares like conical flasks, beakers, Petri dishes, pipettes, oils and powders and similar substances can be sterilized using this technique.

2. DIRECT FLAMING / INCINERATION

Showing the objects or equipments directly to the flame leads the sterilization. This is also called as incineration. The inoculation needles, spread rods, forceps can be sterilized using this technique. It is used for the disposal / sterilization of infected lab animals.

. ADVANTAGES OF HEAT STERILIZATION

a. Sterilization is very effective
b. Heat deliver system can be monitored effectively with various controls like pressure gauge, temperature meters etc
c. Established quality control methods available

. DISADVANTAGES

a) Steam impermeable materials like fats, oils and powders can not be sterilized by autoclaving.
b) Heat sensitive materials like Serum, Antibiotics, Plastic materials, Vaccines and Rubbers can not be sterilized
c) Presence of organic matters interfere with effective sterilization
d) Dangers of explosion can not be sterilized by heat

. LOW TEMPERATURE

Temperature below the optimum level always inhibit the growth and metabolism of microorganisms. Low temperature is used mainly as a mode for microbial preservation. This is only microbisatic not microbicidal.

1. Refrigeration temperature: stored at a temperature of 4°C to 7°C in Refrigerators
2. Deep freezing: stored at a temperature of -20°C to -70°C in Deep Freezers
3. Liquid Nitrogen: stored at a temperature of -196°C

10.2.1. REFRIGERATION

Refrigeration will slow down and inhibit the growth of most microbes but it will not kill them. Note: Some spoilage germs and psychrophiles can continue to replicate at cooler temperatures. Organisms can be maintained viable at -80^{o} C if suspended in glycerol.

10.2.2. DESICCATION

Desiccation of microbes is a very useful means of food preservation and to control the growth of spoilage germs and pathogens. Foods that have a high water activity are most subject to spoilage and typically must be refrigerated or frozen. Numerous foods are preserved by adding salt or sugar to decrease the water activity of the foods. This process

creates hypertonic conditions and causes water to leave bacterial cells (plasmolyze). Salting of foods does not protect against all potential pathogens. Many fungi are halophilic as is *Staphylococcus aureus*, a common source of bacterial food poisoning.

. Radiation

One of the most controversial areas of microbial control involves the use of radiation. The controversy largely results from a lack of understanding of the different types and uses of radiation. The effects of types of radiation depend on three important factors: Time (of exposure), Distance (from the source) , Shielding (how penetrating is the radiation). Irradiation of various food has been used in the U.S since the 1960's and has been used to sterilize foods such as herbs and spices.

Microwaves, ultra violet rays, gamma rays and electrons have the power to kill the microorganisms. Among them, UV light and gamma rays are commonly used for sterilization.

10.3.1. NONIONIZING RADIATION

Includes microwaves and ultra violet radiation. Microwaves are not particularly antimicrobial in and of themselves, rather the killing effect of microwaves are largely due to the heat that they generate. Microwaves are not recommended for cooking large volumes or thick cuts of meat as the heat may not penetrate the foodstuffs sufficiently. Ex: UV rays.

UV radiation is of short wavelength, between 220 and 300 nm and is not very penetrating. UV can be stopped by glass, a sheet of paper, or the top layers of your skin. UV rays can kill exposed microbes by causing damage to their DNA. UV radiation causes T=T thymine dimers. It affects the DNA replication. UV radiation is useful for the disinfection of exposed surfaces such as laboratory hoods. However, the usefulness of UV radiation is limited by the fact that certain microbes possess DNA repair mechanisms and can recover after exposure to this kind of radiation. In addition, UV light does not penetrate organisms well that are protected in mucus or debris. The germicidal effect of UV is higher in 265 nm wavelength.

Laminar air flow chamber is used in this case. Its a clean bench used to handle the microbes in a microbe free environment. It has two methods of sterilization. Radiation by UV and filtration by HEPA filters (High efficiency particulate filters), a microbe free air will be present in side.

. IONIZING RADIATION

Includes gamma rays and X rays which are highly penetrating to cells and tissues and have potent antimicrobial effects. Ionizing radiation knock out electrons from the particles and ionizes them. They create free H radicals, hydroxyl radicals and some peroxidases which in turn causes different kinds of intracellular damages. These free radicals can cause irreversible breaks in DNA, proteins and enzymes. Ex: **Gamma rays.**

γ-rays produced from ^{60}Co and ^{137}Cs are useful for sterilization of the plastic wares, gloves, heat labile plastic containers, poly bags, tissue grafts, plastic syringes etc. In few cases, foods are also sterilized by γ-rays.

Radiation is currently used for sterilization by the medical supply and food industries. Irradiation is the only effective means known to eliminate E. coli 0157 from meat. This process can also eliminate the food pathogens, *Listeria , Campylobacter* and *Salmonella*.

. FILTRATION

Heat is the most common and effective way of sterilization but some heat sensitive liquids and chemicals cannot be sterilized by heat. An alternate technique for the sterilization is by filters. A filter is too small for a passage of microorganisms but large enough for passage of liquids.

Filter sterilization is commonly employed for substances that cannot tolerate heat. Membrane filters with pore sizes between 0.2-0.45 μm are commonly used to remove particles from solutions that can't be autoclaved. Membrane filtration of beer eliminates spoilage germs and pasteurization is no longer needed. Filtered beer is permitted to be sold as "draft beer." Submicron filters are also being marketed for removal of protozoan cysts from drinking water. The mean pore size in bacteriological filters ranges from one to several micrometers. Filters are available in several grades based on average size of pores. Filters can act as mechanical sieves. Other than porosity, the other factors such as electric charge of the filter, electrical charge carried out by the organism and the nature of the fluid become filtered can influence the efficiency of filtration. There are different types of filters based on the material of the filter pad. They are as follows:

1. **Pyrex glass or sintered glass filters**

Filter glass made with powdered glass disc with pore size of 0.5 to 2 μm are used as filters. These discs are fit into funnels and used for filtration. These are used by suction pumps for faster flow rate.

2. **Seitz filter**

The filter mat is made up of asbestos – cellulose mixture.

3. **Berkfield filter**

Diatomaceous earth is used.

4. **Chamberland filter**

Porcelain filter

5. **Membrane filters**

They are composed of polymers with high tensile strength such as cellulose acetate, cellulose nitrate or polysulfonate, manufactured in such a way that they contain a large number of tiny holes. By adjusting the polymerization conditions during manufacture, the size of holes in the membrane can be precisely controlled. They trap the microbes on the surface and allow the microbe free solution to pass. Also called as **molecular filters**. The pore size ranges from 0.01-10 μm. It is used extensively to sterilize fluid materials.

. OVERVIEW OF PHYSICAL AGENTS FOR MICROBIAL CONTROL

S.No	Method	Recommended uses	Limitations
1	Moist heat (Autoclave)	Sterilizing instruments, treatment trays, media and other liquids	Infective against organisms in materials impervious to steam
2	Free flowing steam / boiling water	Destruction of non spore forming pathogens; sanitation of bedding, clothing and dishes	Cannot be guaranteed to produce sterilization on one exposure
3	Dry heat (Hot air oven)	Sterilizing materials impermeable to or damaged by moisture. Ex: oil, glass, sharp instruments, metals	Destructive to materials which cannot withstand high temperatures for long periods
4	Incineration	Disposal of contaminated objects that cannot be reused.	Size of incinerator must be adequate to burn largest load promptly and completely
5	Radiation (UV - Ultra violet rays)	Control of airborne infection and disinfection of surfaces	Must be absorbed to be effective (does not pass through transparent glass or opaque objects), has lesser penetration, irritating to eyes and skin.
6	X-rays, gamma rays and cathode radiation	Sterilization of heat sensitive surgical materials and other medical devices	Expensive and requires special facilities for use
7	Filtration		
	a) Membrane filters	Sterilization of heat sensitive biological fluids	Fluid must be relatively free of suspended particulate matter
	b) Fibre glass filters (HEPA)	Air disinfection	Expensive

CHEMICAL AGENTS FOR MICROBIAL CONTROL

Although objects are sometimes disinfected with physical agents, chemicals are more often employed in disinfection and antisepsis. Many groups of chemicals are able to inhibit the growth and metabolic activity of microorganisms. An efficient chemical agent should satisfy maximum of the following characters:

a. Antimicrobial activity
b. Solubility
c. Stability
d. Availability
e. Noncorroding and nonstaining
f. Deodorizing ability
g. Homogeneity
h. Nontoxicity to humans and other animals
i. Capacity to penetrate through surfaces
j. Detergent capacities (cleaning and cleansing effect)
k. Non combination with extraneous organic matter

Here are the list of **MAJOR GROUPS OF CHEMICAL ANTIMICROBIAL AGENTS** that are often used for chemical means of microbial control,

1. Phenol and phenolic compounds
2. Alcohols
3. Halogens
4. Heavy metals and their compounds
5. Dyes
6. Detergents
7. Quaternary ammonium compounds
8. Aldehydes
9. Gaseous agents

. PHENOL & PHENOLIC COMPOUNDS

Phenol was the first widely used antiseptic and disinfectant. In 1867 Joseph Lister employed it to reduce the risk of infection during operations. Today phenol and phenolics (phenol derivatives) such as cresols, xylenols, and orthophenylphenol are used as disinfectants in laboratories and hospitals. The commercial disinfectant Lysol is made of a mixture of phenolics.

Phenolics act by denaturing proteins and disrupting cell membranes. They have some real advantages as disinfectants: phenolics are tuberculocidal, effective in the presence of organic material, and remain active on surfaces long after application. However, they do have a disagreeable odor and can cause skin irritation.

Alcohols

Alcohols are among the most widely used disinfectants and antiseptics. They are bactericidal and fungicidal but not sporicidal; some lipid-containing viruses are also destroyed. The two most popular alcohol germicides are ethanol and isopropanol, usually used in about 70 to 80% concentration. They act by denaturing proteins and possibly by dissolving membrane lipids. 10 to 15 minute soaking is sufficient to disinfect thermometers and small instruments.

. Halogens

A halogen is any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VIIA of the periodic table. They exist as diatomic molecules in the free state and form saltlike compounds with sodium and most other metals. The halogens iodine and chlorine are important antimicrobial agents.

Iodine is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. At higher concentrations, it may even kill some spores. Iodine often has been applied as **tincture of iodine**, 2% or more iodine in a water-ethanol solution of potassium iodide. Although it is an effective antiseptic, the skin may be damaged, a stain is left, and iodine allergies can result. More recently iodine has been complexed with an organic carrier to form an **iodophor.** Iodophors are water soluble, stable, and nonstaining, and release iodine slowly to minimize skin burns and irritation. They are used in hospitals for preoperative skin degerming and in hospitals and laboratories for disinfecting. Some popular brands are Wescodyne for skin and laboratory disinfection and Betadine for wounds.

Chlorine is the usual disinfectant for municipal water supplies and swimming pools and is also employed in the dairy and food industries. It may be applied as chlorine gas, sodium hypochlorite, or calcium hypochlorite, all of which yield hypochlorous acid (HClO) and then atomic oxygen. The result is oxidation of cellular materials and destruction of vegetative bacteria and fungi, although not spores. Chlorine is also an excellent disinfectant for individual use because it is effective, inexpensive, and easy to employ. Small quantities of drinking water can be disinfected with halazone tablets. Halazone (parasulfone dichloramidobenzoic acid) slowly releases chloride when added to water and disinfects it in about a half hour. It is frequently used by campers lacking access to uncontaminated drinking water. Chlorine solutions make very effective laboratory and household disinfectants.

. Heavy metals

For many years the ions of heavy metals such as mercury, silver, arsenic, zinc, and copper were used as germicides. More recently these have been superseded by other less toxic and more effective germicides (many heavy metals are more bacteriostatic than bactericidal).

There are a few exceptions. 1% solution of silver nitrate is often added to the eyes of infants to prevent ophthalmic gonorrhea (in many hospitals, erythromycin is used instead of silver nitrate because it is effective against *Chlamydia* as well as *Neisseria*). Silver sulfadiazine is used on burns. Copper sulfate is an effective algicide in lakes and swimming pools.

Heavy metals combine with proteins, often with their sulfhydryl groups, and inactivate them. They may also precipitate cell proteins.

. Dyes

Commonly used dyes are Triphenyl Methane and Acridine orange. Gram positive are more sensitive to dyes. Dyes are used in selective media preparation. Eg. Brilliant green, Crystal violet inhibit Gram positive

. Quaternary disinfectants

Detergents [Latin *detergere,* to wipe off or away] are organic molecules that serve as wetting agents and emulsifiers because they have both polar hydrophilic and nonpolar hydrophobic ends. Due to their amphipathic nature, detergents solubilize otherwise insoluble residues and are very effective cleansing agents. They are different than soaps, which are derived from fats.

Synthetic detergents donot form precipitates in alakaline or acid water. Its extensively used in laundries, dish wash powders, shampoos and other washing preparations. They are of two types. Anionic and cationic detergents.

Although anionic detergents have some antimicrobial properties, only cationic detergents are effective disinfectants. The most popular of these disinfectants are **quaternary ammonium compounds** characterized by a positively charged quaternary nitrogen and a long hydrophobic aliphatic chain. Quternary ammonium compounds have a wide range of damaging properties on microorganisms like denaturation of proteins, interference with glycolysis and membrane transport. They disrupt microbial membranes. They are used as skin disinfectants, as a preservative in ophthalmic solutions and in cosmetic preparations. Also used in floors, walls and other surfaces in hospital. They are also used to sanitize food and certain equipments in food processing plants.

. Aldehydes

Both of the commonly used aldehydes, formaldehyde and glutaraldehyde, are highly reactive molecules that combine with nucleic acids and proteins and inactivate them, probably by crosslinking and alkylating molecules. They are sporicidal and can be used as chemical sterilants. Formaldehyde is usually dissolved in water or alcohol before use. 2% buffered solution of glutaraldehyde is an effective disinfectant. It is less irritating than formaldehyde and is used to disinfect hospital and laboratory equipment. Glutaraldehyde usually disinfects objects within about 10 minutes but may require as long as 12 hours to destroy all spores.

. Gaseous agents

Many heat-sensitive items such as disposable plastic Petri dishes and syringes, heart-lung machine components, sutures, and catheters are now sterilized with ethylene oxide gas.

Ethylene oxide is both microbicidal and sporicidal and kills by combining with cell proteins. It is a particularly effective sterilizing agent because it rapidly penetrates packing materials, even plastic wraps. Sterilization is carried out in a special ethylene oxide sterilizer, very much resembling an autoclave in appearance, that controls the EtO concentration, temperature, and humidity.

Betapropiolactone (BPL) is occasionally employed as a sterilizing gas. In the liquid form it has been used to sterilize vaccines and sera. BPL decomposes to an inactive form after several hours and is therefore not as difficult to eliminate as EtO. It also destroys microorganisms more readily than ethylene oxide but does not penetrate materials well and may be carcinogenic. For these reasons, BPL has not been used as extensively as EtO. Recently vapor-phase hydrogen peroxide has been used to decontaminate biological safety cabinets.

. OVERVIEW OF CHEMICAL AGENTS USED FOR MICROBIAL CONTROL

S. No	Chemical	Use	Mode of action
1	Alcohol	Skin, medical instruments, food, dairy instruments, lab surface	Lipid solvent and protein denaturant
2	Phenol compounds	Soaps, lotions, cosmetics, body deodorants	Disrupt cell membrane
3	Cationic detergents	Soap, lotion	Pospholipid interaction
4	Quaternaries	Skin disinfection	Not sporicidal
5	3% hydrogen peroxide	Skin	Oxidizing agent
6	Iodine	Skin	Iodinates tyrosine residues; oxidizing agent
7	Silver nitrate	Treating burns and in eyes of new born to prevent blindness from *Nesseria gonorrhea*	Protein precipitant
8	Cationic detergents	Medical instruments, food, dairy instruments, lab surface	Interact with phospholipids
9	Chlorine	Disinfectant for food and dairy industry, water supplies	Oxidizing agent
10	Copper sulphate	Algicide in swimming pools	Protein precipitant
11	Ethylene oxide gas	Sterilant for temperature sensitive lab material like plastics	Alkylating agent
12	Formaldehyde	3-8% used as surface disinfectant; 37% (referred as formalin) as preservatives	Alkylating agent

13	Gluteraldehyde	Sterilizing instruments and fumigation	Alkylating agent
14	Mercuric chloride	For laboratory surface, plant samples	Protein denaturation
15	Salt and sugar solutions	Preservative	Osmotic pressure
16	Antibiotics		
	a) Penicillin, Ampicillin	Effective against Gram positive bacteria	Inhibit cell wall synthesis
	b) Streptomycin	Effective against Gram positive and Gram negative bacteria	Inhibits protein synthesis