Sterilization & Disinfection

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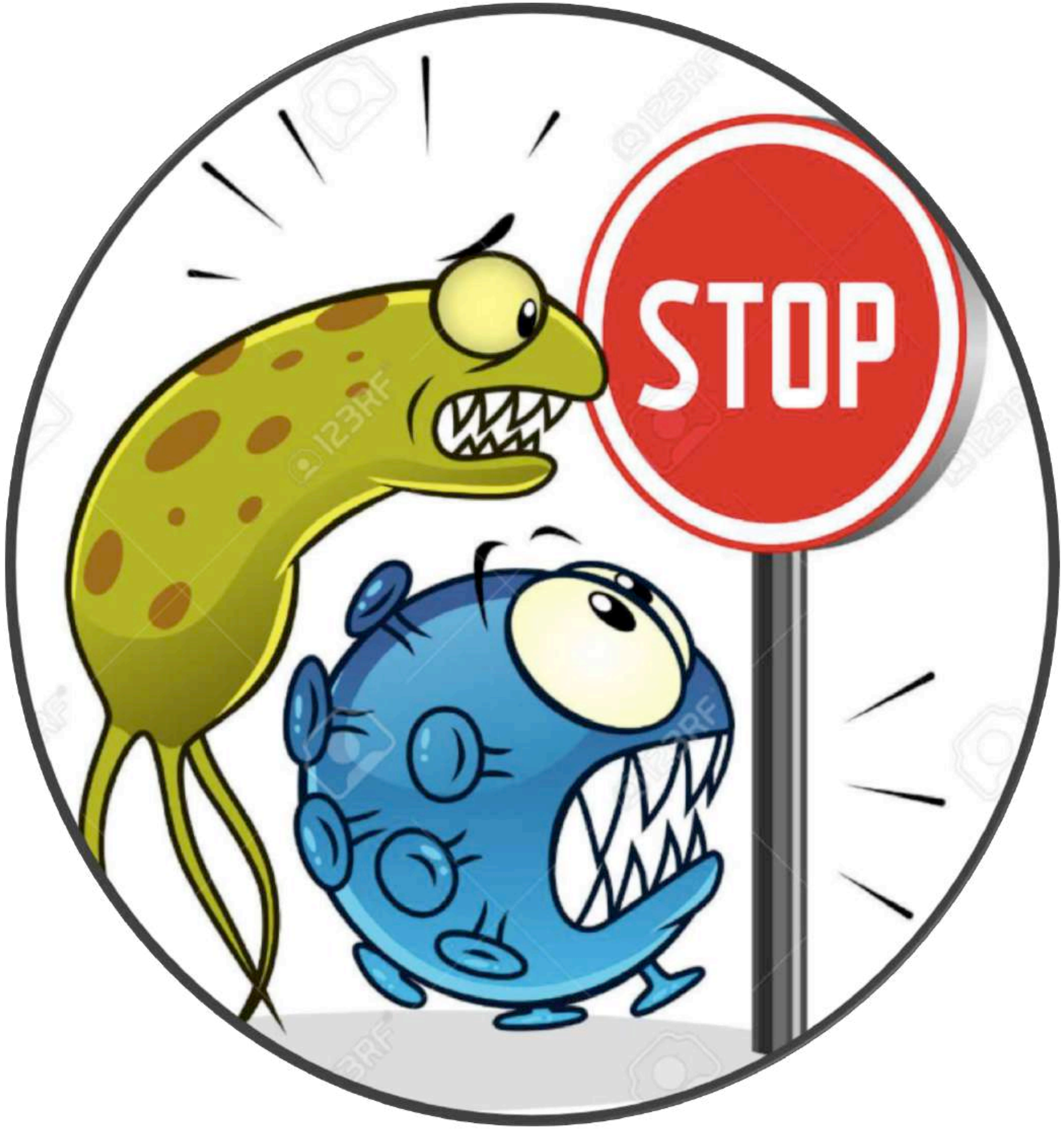
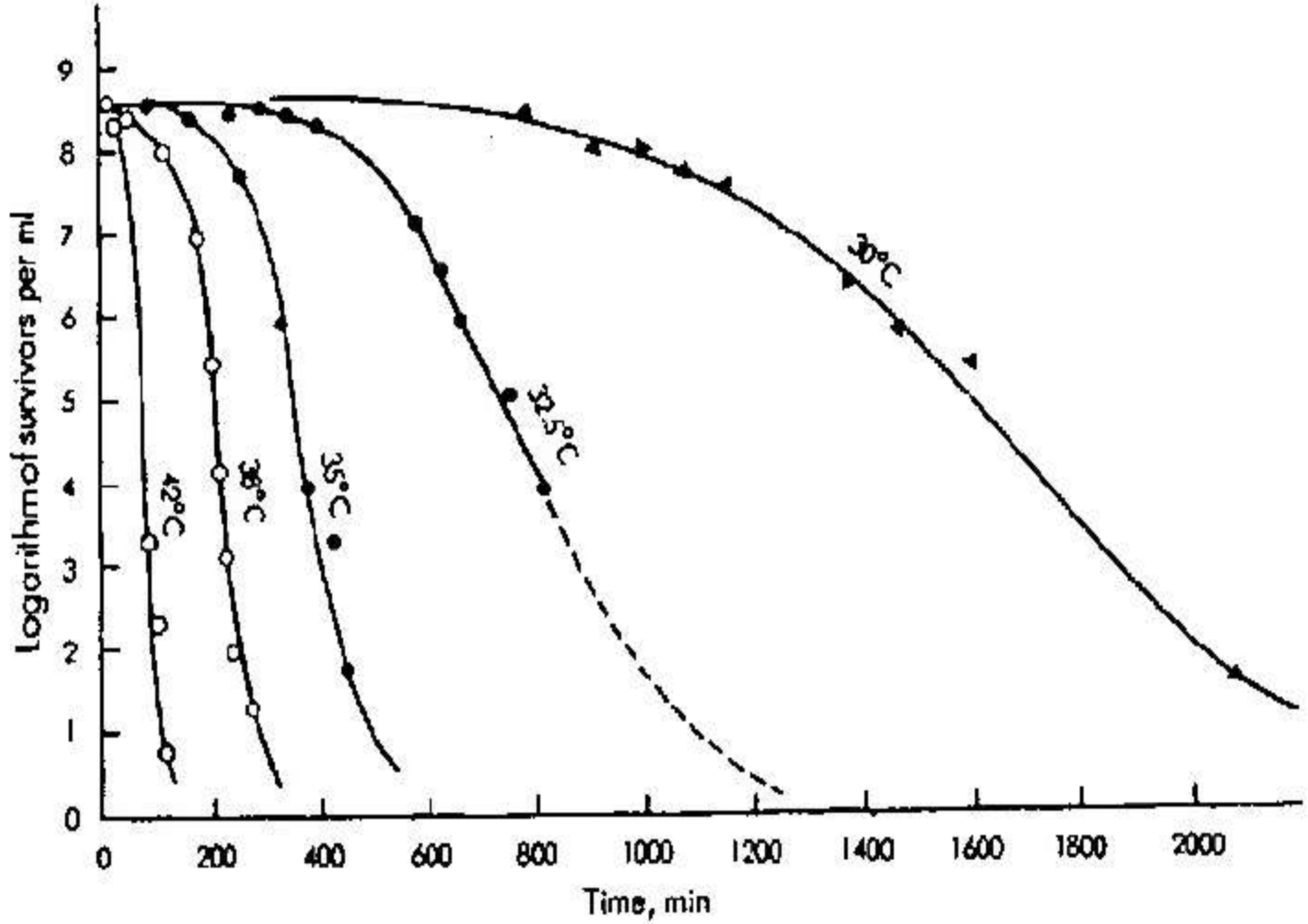


Introduction

- Sterilization
- Disinfectant
- Antispetic
- Sanitizer
- Microbicide / Germicide
- Microbistatic
- Antimicrobial Agent

Conditions influencing antimicrobial action

- Environment
- Kind of microorganism
- Physiological state of cell



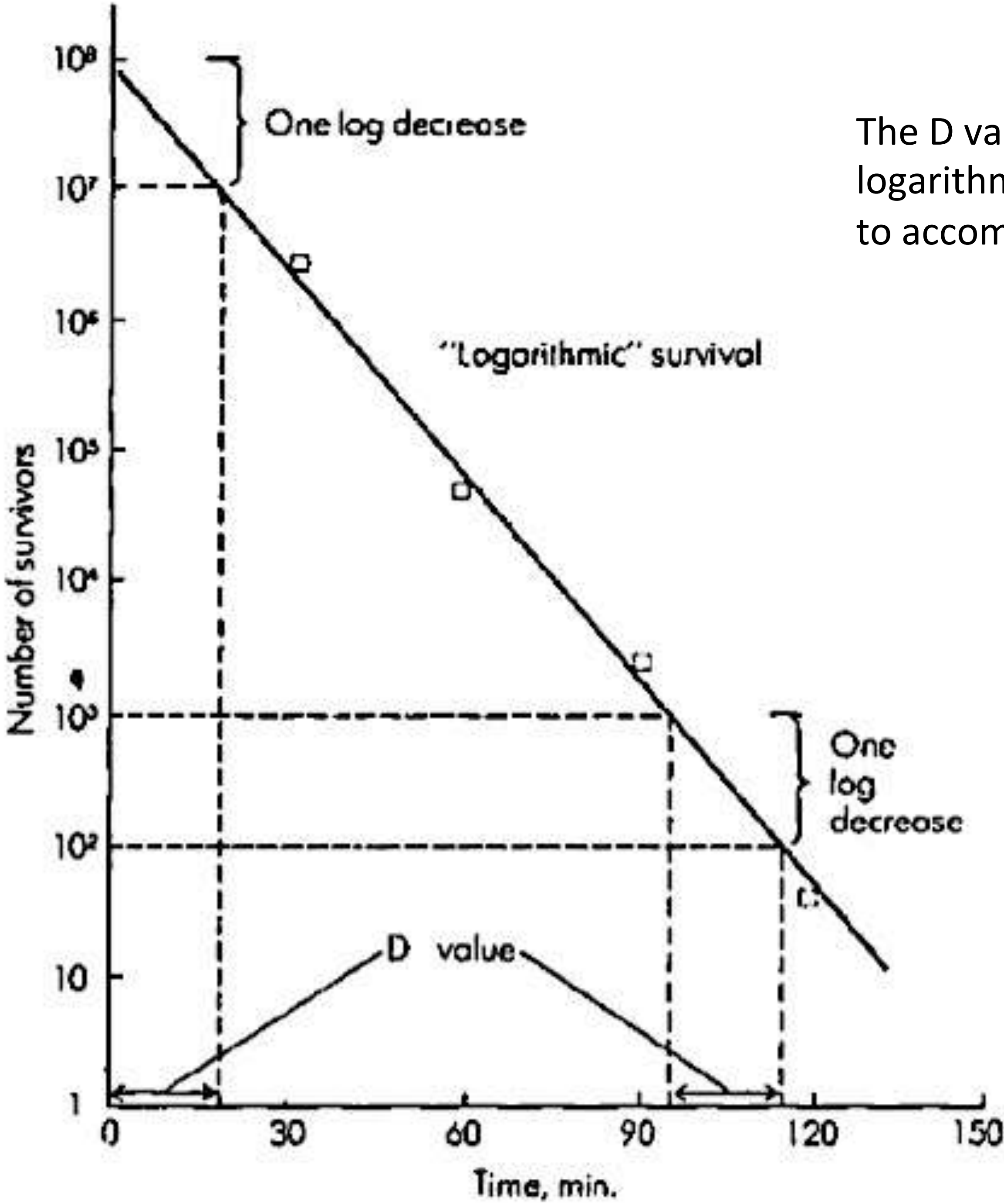
1. Physical Agents & Methods

- Temperature
- Filtration
- Radiations

1.1 High temperature

- Microorganisms can grow over a wide range of temperatures
  - Psychrophiles
  - Thermophiles
- Every type has an optimum, minimum, and maximum growth temperature.
- Temperatures above the maximum generally kill, while those below the minimum usually produce stasis.

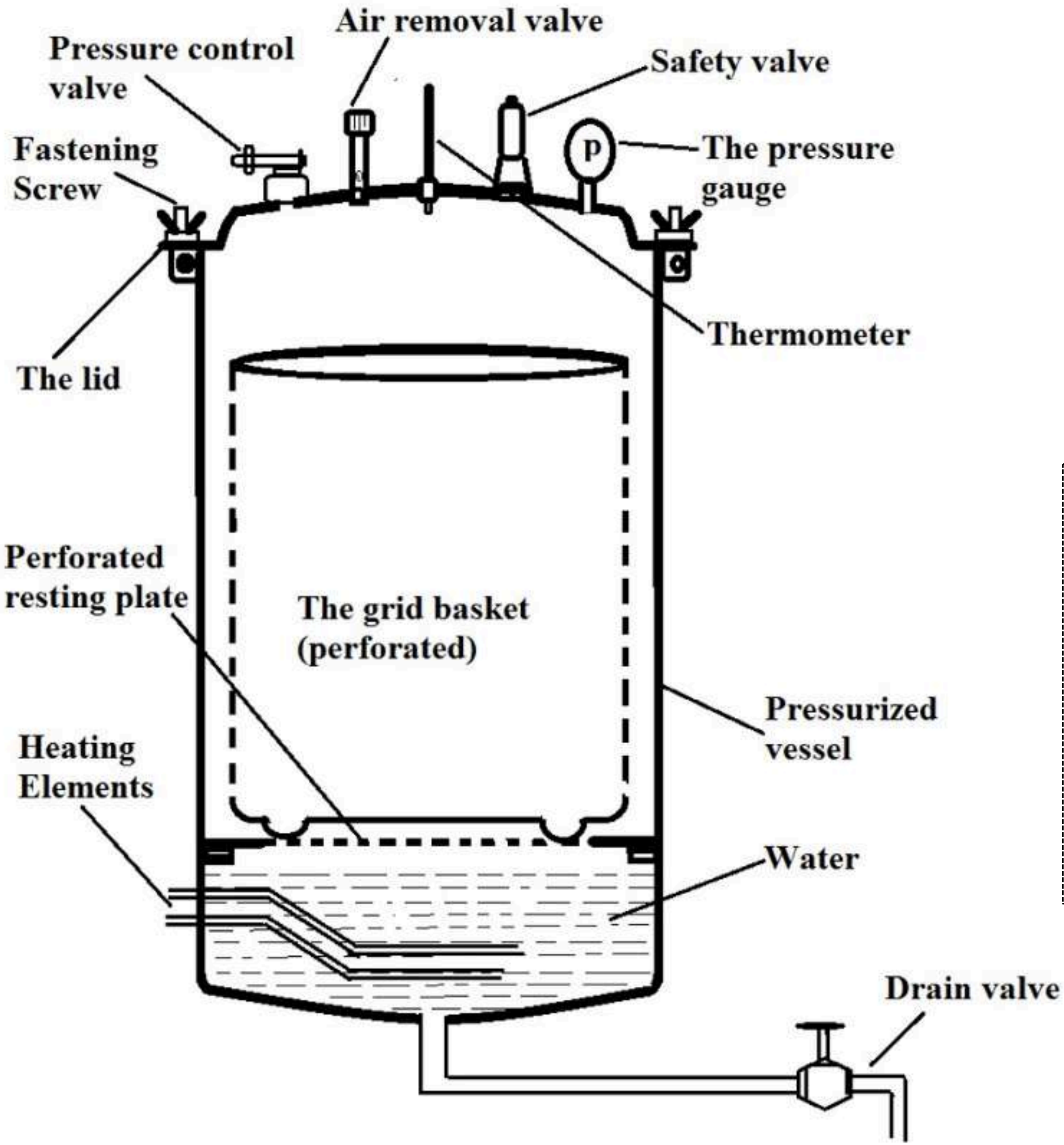
- **Thermal Death Time (TDT)**
  - the shortest period of time to kill a suspension of bacteria (or spores) at a specific temperature.
- **Thermal Death Point (TDP)**
  - the lowest temperature required to kill a suspension of bacteria (or spores) in a specified time.
- **Decimal Reduction Time (D-value)**
  - This is the time in minutes to reduce the population by 90 percent, or stated differently, it is the time in minutes for the thermal death-time curve to pass through one log cycle



The D value is independent of time when the response is logarithmic, that is, when the same length of time is required to accomplish any given log decrease in number of survivors.

a. Moist Heat

- High temperature combined with moisture
- Heat in the form of saturated steam under pressure
  - Temperature above BP of water
- It has the advantages of rapid heating, penetration, and moisture in abundance, which facilitates the coagulation of proteins
- Vegetative cells sensitive compared to spores → water activity
- Rapid and Effective
- Practical application
  - Sterilization of many media, solutions, discarded cultures, and contaminated materials



Steam under pressure –
Autoclave: 15 psi, 121°C, 15 min

Indicators can also be used.

In the operation of an autoclave it is absolutely essential that the air in the chamber be completely replaced by saturated steam.

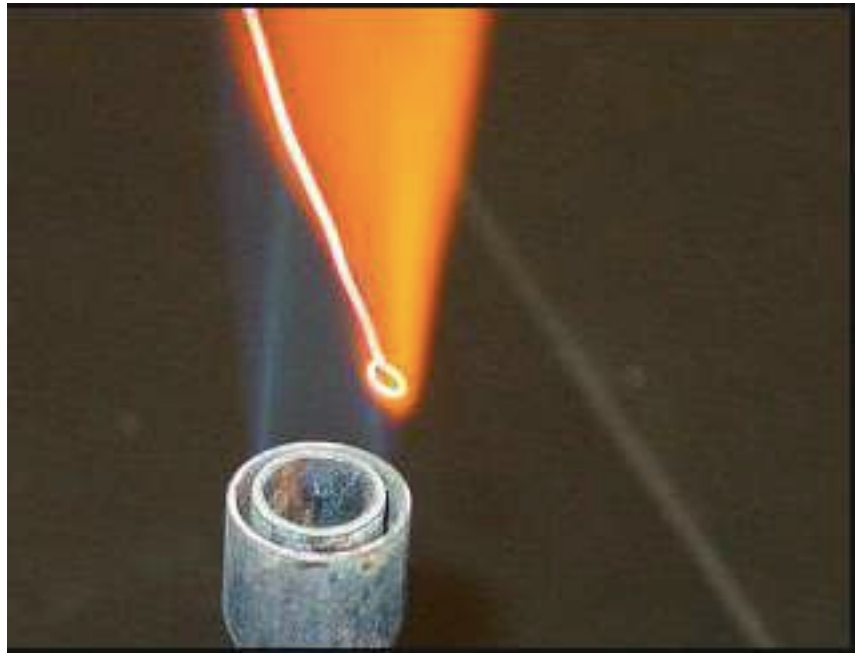
If air is present, it will reduce the temperature obtained within the chamber substantially below.

It is not the pressure that kills the organisms but the temperature of the steam.

b. Dry Heat

- Hot-air sterilization
  - When moist heat is either undesirable or will make direct contact with the material
- Instrument: “Hot-air Oven”
  - Laboratory glasswares, oils, powders
  - 160°C for 2 hrs
- Kills by oxidizing the chemical constituents of microorganisms
- Less effective than moist heat
  - Spores of *Clostridium botulinum* are killed in 4 to 20 min by moist heat at 120°C, whereas a 2-h exposure to dry heat at the same temperature is required.
  - Spores of *B. anthracis* are destroyed in 2 to 15 min by moist heat at 100°C, but with dry heat 1 to 2 h at 150°C is required to achieve the same result

c. Incineration



- Destruction of microorganisms by burning
  - Laboratory : the transfer needle / nichrome loop / spreader is introduced into the flame of the Bunsen burner **(flame sterilization)**
    - When the transfer needle is sterilized, care should be exercised to prevent spattering, since the droplets which fly off are likely to carry viable organisms.
  - Destruction of carcasses, infected laboratory animals, and other infected materials to be disposed of.
    - Special precautions need to be taken to ensure that the exhaust fumes do not carry particulate matter containing viable microorganisms into the atmosphere.

1.2 Filtration

- Variety of filters have been available to remove microorganisms from liquids or gases.
- These filters are made of different materials—
  - Asbestos pad in the Seitz filter
  - Diatomaceous earth in the Berkefeld filter,
  - Porcelain in the Chamberland-Pasteur filter, and
  - Sintered glass disks in other filters

Berkefeld

Sinter glass filter

Seitz filter

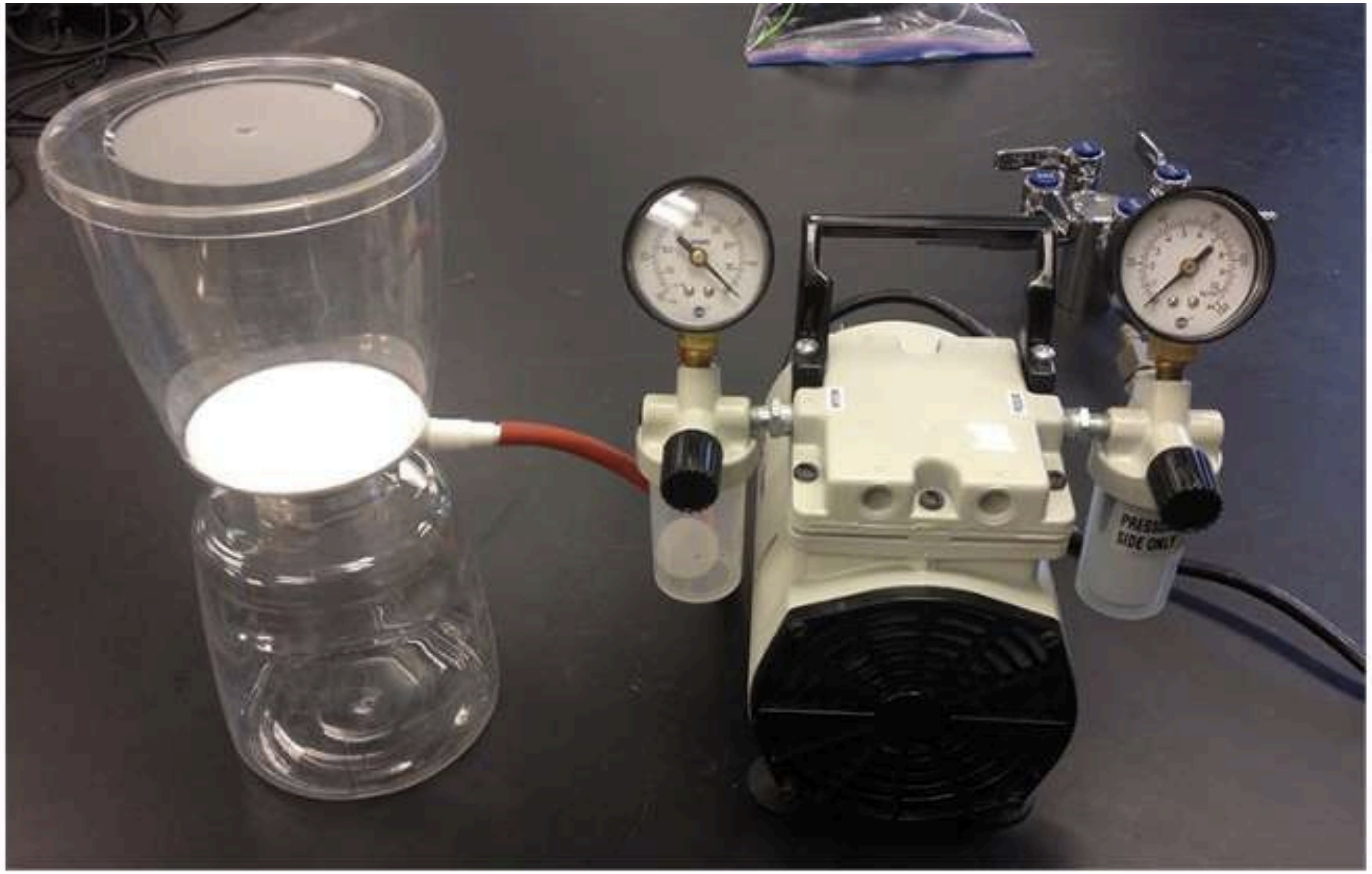
- The mean pore diameter in these bacteriological filters ranges from one to several micrometers
    - Most filters are available in several grades, based on the average size of the pores.
- However, it should be understood that these filters do not act as mere mechanical sieves; porosity alone is not the only factor preventing the passage of organisms.
- Other factors, such as the electric charge of the filter, the electric charge carried by the organisms, and the nature of the fluid being filtered, can influence the efficiency of filtration.

a. Membrane Filtration

- **Membrane or molecular filter**
  - Pores are of a uniform and specific predetermined size.
  - Composed of biologically inert cellulose esters.
  - Circular membranes of about 150-um thickness and contain millions of microscopic pores of very uniform diameter
    - Filters of this type can be produced with known porosities ranging from approximately 0.01 to 10 um.
- Membrane filters are used extensively in the laboratory and in industry to sterilize fluid materials.
- They have been adapted to microbiological procedures for the identification and enumeration of microorganisms from water samples and other materials

Membrane Filtration Assembly

Syringe filters

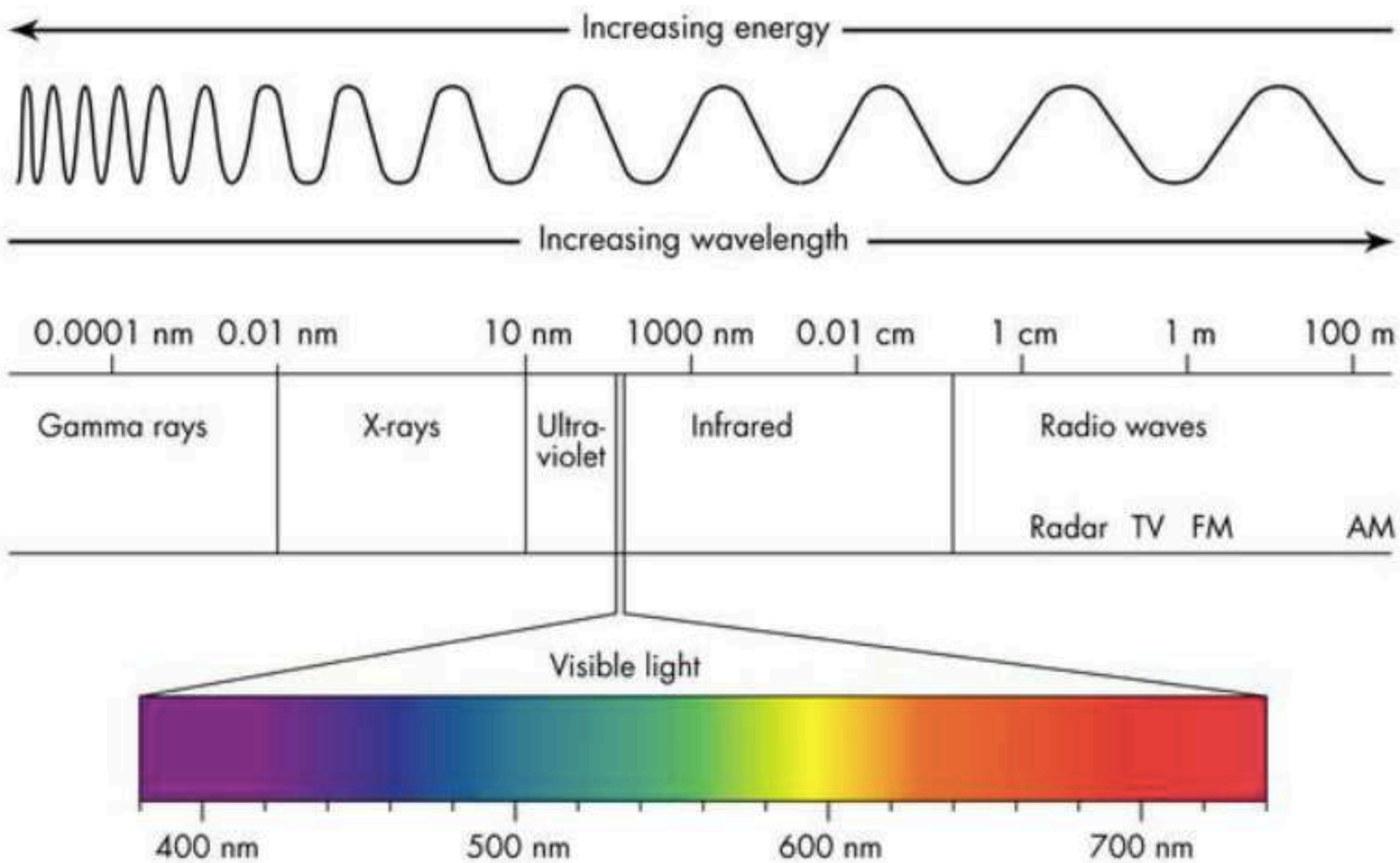
It is customary to force the fluid through the filter by applying a negative pressure to the filter flask by use of a vacuum or water pump or to impose a positive pressure above the fluid in the filter chamber, thus forcing it through. Upon completion of filtration, precautions must be taken to prevent contamination of the filtered material when it is transferred to other containers.

b. HEPA Filters

- **High-efficiency particulate air (HEPA)** filters
  - Deliver clean air to an enclosure such as a cubicle or a room.
  - This type of air filtration together with a system of laminar airflow is now used extensively to produce dust- and bacteria-free air.

1.3 Radiations

- Electromagnetic radiation
- Dual nature
- Different energies
- Sterilizing agents



a. UV

- Range from 150 to 3900 Å.
  - Wavelengths around 2650 Å have the highest bactericidal efficiency
  - Sunlight : 2670 to 3900 Å
  - UV lamps/ germicidal lamp : 2600 to 2700 Å
- Non-ionizing and non-penetrating (less energy)
  - Even a thin layer of glass filters off a large percentage of the light
  - Direct exposure required
- Absorbed by many cellular materials but most significantly by the nucleic acids, where it does the most damage

b. X-rays

- Ionizing radiations
  - Energy to knock electrons away from molecules and ionize them
  - Create free hydrogen radicals, hydroxyl radicals, and some peroxides
  - Cause different kinds of intracellular damage → nonspecific effects
- Penetrating power
- They are impractical for purposes of controlling microbial populations
  - They are very expensive to produce in quantity
  - They are difficult to utilize efficiently, since radiations are given off in all directions from their point of origin.

c. Gamma Rays

- Ionizing radiations
- Great penetration into matter (high energy), and they are lethal to all life, including microorganisms
- Produced during radioactive decay
- Used in commercial sterilization of materials of considerable thickness or volume, e.g. packaged foods and medical devices.

d. Cathode Rays/Electron Beam Radiation

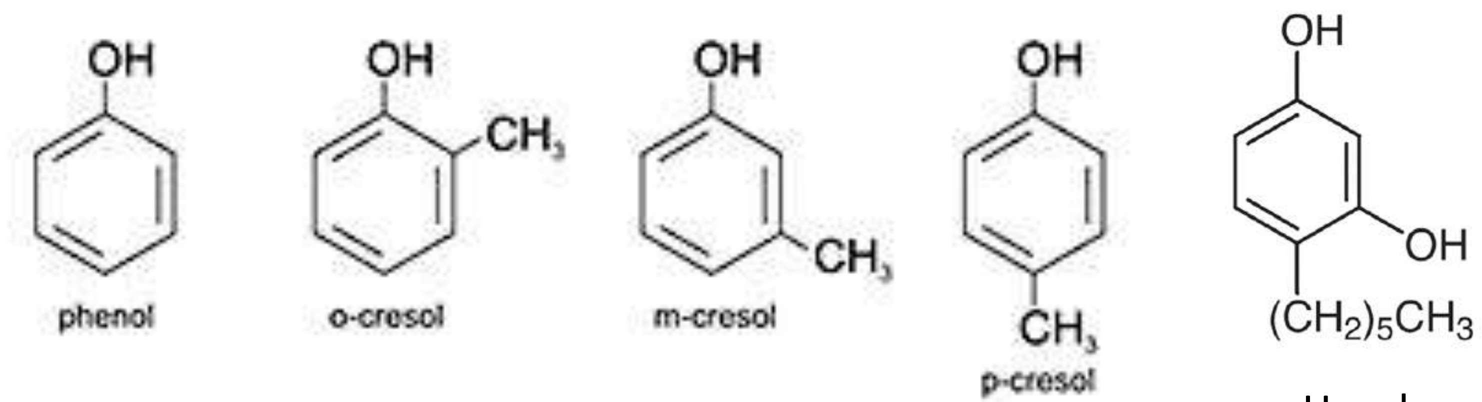
- During a high-voltage potential between a cathode and an anode in an evacuated tube, the cathode emits beams of electrons
  - Special types of equipment have been designed which produce electrons of very high intensities (millions of volts), and these electrons are accelerated to extremely high velocities.
- These intense beams of accelerated electrons are microbicidal
  - Sterilization of surgical supplies, drugs, and other materials.
  - Material can be sterilized after it has been packaged (the radiations penetrate the wrappings) and at room temperature.
- Sterilization is accomplished on very brief exposure

2. Chemical Agents

- Phenols & phenolic compounds
- Alcohols
- Halogens
- Heavy metals
- Dyes
- Detergents
- Quaternary ammonium salts
- Aldehydes
- Gaseous agents

2.1 Phenols & phenolic compounds

- Earlier years, used to decrease infection of surgical incisions & wounds
- Effective disinfectant
  - 5% solution → rapidly kills vegetative cells (spores more resistant)
- Antimicrobial activity is enhanced by the addition of chemical substitutions in the phenol ring structure



Hexylresorcinol

- Phenolic substances may be either bactericidal or bacteriostatic, depending upon the concentration used.
  - Bacterial spores and viruses are more resistant than are vegetative cells.
- Some phenolics are highly fungicidal.
- 2-5% solution as disinfectant for sputum, urine, feces, contaminated instrument, utensils
- Antimicrobial activity reduced at alkaline pH, presence of organic material, low temperature, presence of soap
- Derivatives of phenol diluted in detergents or some other carrier find use in many commercial antiseptic and disinfectant preparations.
  - Example: o-phenylphenol
    - Combination of compounds of this class with detergents results in products with good disinfectant as well as detergent properties.

- Mode of action
  - Exposure of microbial cells to phenolic compounds produces a variety of effects.
  - Depending upon the concentration of the phenolic compound to which microbial cells were exposed
    - disruption of cells
    - precipitation of cell protein
    - inactivation of enzymes
    - leakage of amino acids from the cells
    - physical damage to the membrane structures in the cell surface → lethal effect

2.2 Alcohols

- Alcohol is effective in reducing the microbial flora of skin and for the disinfection of clinical oral thermometers.
  - Alcohol concentrations above 60% are effective against viruses; however, the effectiveness is influenced by the amount of extraneous protein material in the mixture → reacts with the alcohol and thus protects the virus.
- **Ethyl alcohol / Ethanol** in concentrations between 50 and 90%, is effective against vegetative or non-spore forming cells.
  - For practical application a 70% concentration of alcohol is generally used.
  - Ethyl alcohol cannot be relied upon to produce a sterile condition.
  - Concentrations which are effective against vegetative cells are practically inert against bacterial spores.

- **Methyl alcohol / Methanol** is less bactericidal than ethyl alcohol
  - it is highly poisonous.
  - Even the fumes of this compound may produce permanent injury to the eyes, and is not generally employed for the destruction of microorganisms.

- **The higher alcohols—propyl, butyl, amyl, and others**—are more germicidal than ethyl alcohol.
  - In fact, there is a progressive increase in germicidal power as the molecular weight of alcohols increases
  - Since alcohols of molecular weight higher than that of propyl alcohol are not miscible in all proportions with water, they are not commonly used in disinfectants.
  - Propyl and isopropyl alcohols in concentrations ranging from 40 to 80% are bactericidal for vegetative cells

- Mode of Action.
  - Alcohols are protein denaturants.
  - Alcohols are also solvents for lipids, and hence they may damage lipid complexes in the cell membrane.
  - They are also dehydrating agents.
    - This may account for the relative ineffectiveness of absolute alcohol on "dry" cells; it is possible that very high concentrations remove so much water from the cell that the alcohol is unable to penetrate.
    - The severe dehydration occurring under these conditions would result in a bacteriostatic condition.

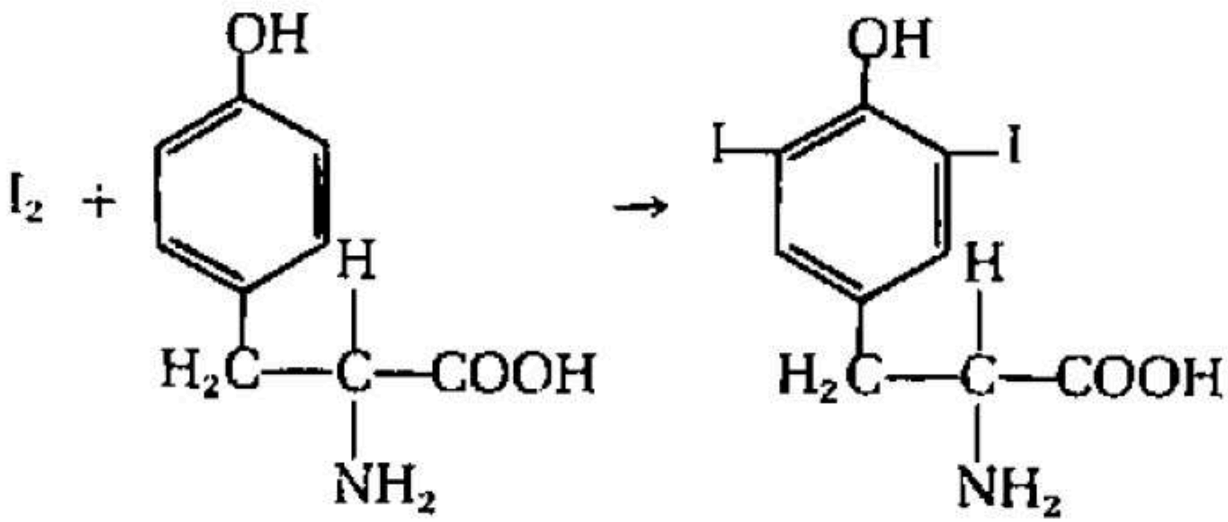
2.3 Halogens

IODINE

- Oldest and most effective germicidal agents → "**tincture of iodine**".
- Pure iodine is a bluish-black crystalline element having a metallic luster.
- Slightly soluble in water, readily soluble in alcohol & aqueous solutions of KI or NaI
  - 2% iodine plus 2% sodium iodide diluted in alcohol
  - 7% iodine plus 5% potassium iodide in 63% alcohol
  - 5% iodine and 10% potassium iodide in aqueous solution
- Also used in the form of **iodophors** (mixtures of iodine with surface-active agents which act as carriers and solubilizers for the iodine)
  - E.g. Polyvinyl pyrrolidone → PVP-I
  - Iodine is released slowly from this complex.

- Iodine is a highly effective bactericidal agent and is unique in that it is **effective against all kinds of bacteria**.
- Iodine also possesses **sporicidal** activity; however, the rate at which the spores are killed is markedly influenced by the conditions under which they are exposed, e.g., amount of organic material and extent of dehydration.
- In addition, it is highly **fungicidal** and is to some extent **virucidal**.
- Iodine solutions are chiefly used for the disinfection of skin, and for this purpose they rank among the best disinfectants.
- Iodine preparations are effective for other purposes, such as disinfection of water, disinfection of air (iodine vapors), and sanitization of food utensils.

- Mode of Action.
  - Iodine is an oxidizing agent
  - Oxidizing agents can irreversibly oxidize and thus inactivate essential metabolic compounds such as proteins with sulfhydryl groups.
  - The action may involve the halogenation of tyrosine units of enzymes and other cellular proteins requiring tyrosine for activity.

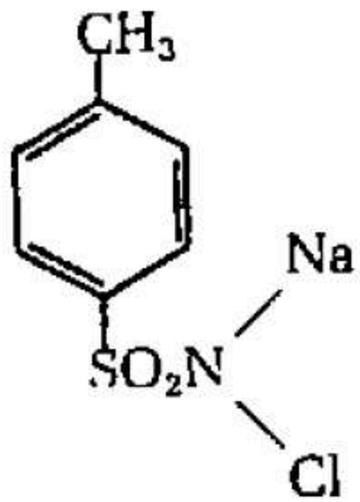


Tyrosine Diiodotyrosine

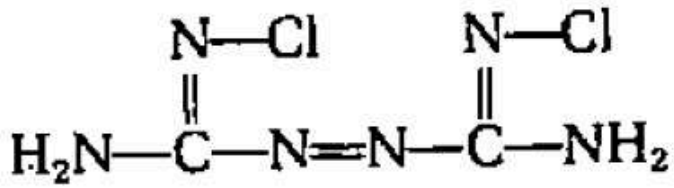
CHLORINE

- Disinfectants - Either in the form of gas or in certain chemical combinations
  - The compressed gas in liquid form is almost universally employed for the purification of municipal water supplies.
  - Chlorine gas is difficult to handle → special equipment to dispense.
    - Hence, its usefulness in the gaseous state is limited to large-scale operations such as water-purification plants, where it is feasible for installing suitable equipment for safe handling.
- There are available many compounds of chlorine → handled more conveniently than free chlorine and equally effective as disinfectants.
  - Hypochlorites
    - Calcium hypochlorite, $Ca(OCl)_2$ (also known as chlorinated lime)
    - Sodium hypochlorite, $NaOCl$

- Chloramines → disinfectants, sanitizing agents, or antiseptics.
  - One or more of the hydrogen atoms in an amino group of a compound are replaced with chlorine.
    - Monochloramine (NH_2Cl)
    - Chloramine-T
    - Azochloramide
  - They are more stable than the hypochlorites in terms of prolonged release of chlorine



Chloramine-T



Azochloramide

- Chlorine compounds have been used to disinfect open wounds, to treat athlete's foot, to treat other infections, and as a general disinfectant.
- Major applications are in water treatment, in the food industry, for domestic uses, and in medicine.
- Products containing calcium hypochlorite are used for sanitizing dairy equipment and eating utensils in restaurants.
- Solutions of sodium hypochlorite of a 1% concentration are used for personal hygiene and as a household disinfectant.
- Higher concentrations of 5 to 12% are also employed as household bleaches and disinfectants and for use as sanitizing agents in dairy and food-processing establishments.

- Mode of Action.
  - The hypochlorous acid formed when free chlorine/hypochlorites/ chloramines is added to water.
  - The hypochlorous acid formed is further decomposed → The nascent oxygen released → strong oxidizing agent, and through its action on cellular constituents, microorganisms are destroyed.

$$Cl_2 + H_2O \rightarrow HCl + HClO \text{ (hypochlorous acid)}$$

$$HClO \rightarrow HCl + O$$

Formed from chlorine, hypochlorites, chloramines

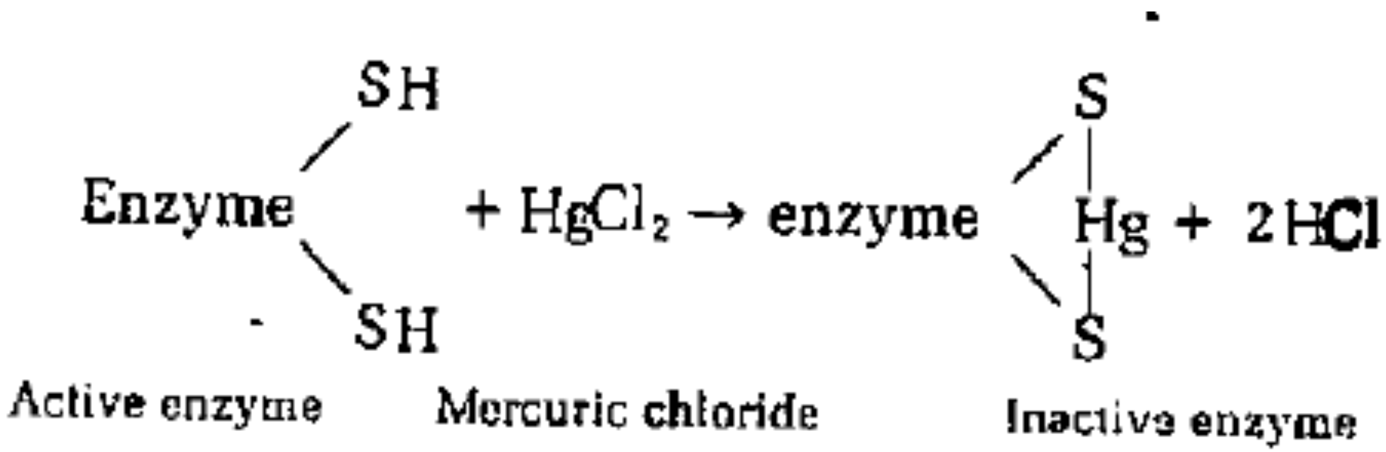
  - The killing of microorganisms → also by direct combination of chlorine with proteins of the cell membranes and enzymes.

2.4 Heavy Metals & Compounds

Table 23-3. Some Compounds of Heavy Metals That Have Antimicrobial Activity

Heavy Metal	Examples of Compounds	Applications
Mercury	Inorganic compounds: Mercuric chloride (bichloride of mercury) Mercurous chloride Mercuric oxide Ammoniated mercury	Bactericidal in dilutions of 1:1,000; limited use because of corrosive action, high toxicity to animals, and reduction of effectiveness in presence of organic material; insoluble compounds, used in ointments as antiseptics
	Organic compounds: Mercurochrome Metaphen Merthiolate Mercresin	Less irritating and less toxic than the inorganic mercury compounds; employed as antiseptics on cutaneous and mucosal surfaces; may be bactericidal or bacteriostatic
Silver	Colloidal silver compounds: Silver nitrate Silver lactate Silver picrate	Consist of protein in combination with metallic silver or silver oxide (colloidal solution); bacteriostatic or bactericidal effect is a function of the free silver ions released from the combination; used as antiseptics, silver nitrate is the most widely used of these compounds, all of which are germicidal and employed as antiseptics in specific conditions; silver nitrate is bactericidal for most organisms at a dilution of 1:1,000; many states require that the eyes of newborns be treated with a few drops of 1% silver nitrate solution to prevent ophthalmia neonatorum, a gonococcal infection of eyes
Copper	Copper sulfate	Much more effective against algae and molds than bacteria; 2 ppm in water sufficient to prevent algal growth; used in swimming pools and open water reservoirs; used in the form of Bordeaux mixture as a fungicide for prevention of certain plant diseases.

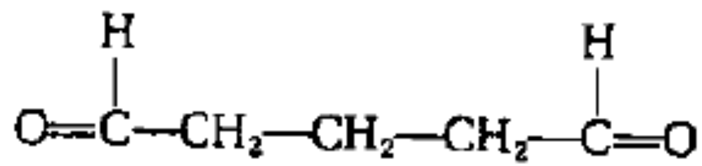
- Mode of Action
  - Combine with cellular proteins and inactivate them
  - Coagulate cytoplasmic proteins, resulting in damage or death to the cell.
    - For example, in the case of mercuric chloride the inhibition is directed at enzymes which contain the sulfhydryl grouping.



  - Heavy metals and their salts are precipitants

2.5 Aldehydes

- General formula RCHO (aldehydes)
- Several of the low-molecular-weight compounds are antimicrobial
- Most effective are formaldehyde and glutaraldehyde.
  - Microbicidal & sporicidal



<u>GLUTARALDEHYDE</u>

- 2% solution exhibits a wide spectrum of antimicrobial activity.
  - It is effective against vegetative bacteria, fungi, bacterial and fungal spores; and viruses.
  - It is used in the medical field for sterilizing urological instruments, lensed instruments, respiratory therapy equipment, and other special equipment.

FORMALDEHYDE (HCHO)

- Gas → stable only in high concentrations and at elevated temperatures.
- Solid → at room temperature (polymerizes forming a solid substance)
  - e.g. paraformaldehyde, a colorless substance which rapidly yields formaldehyde upon heating.
- Liquid → formalin (aqueous solution of 37 to 40% formaldehyde)
- USES
  - Solution for sterilization of certain instruments.
  - Gaseous form can be used for disinfection and sterilization of enclosed areas (fumigation).
    - Cause sterilization, vegetative cells being killed more quickly than spores
    - Formalin and paraformaldehyde as sources of formaldehyde for gaseous disinfection.
    - Humidity and temperature important for microbicidal action of formaldehyde
      - room temperature (22°C) and the relative humidity between 60 to 80 percent.

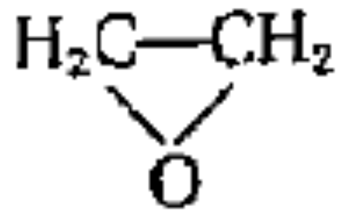
- Disadvantages
  - the limited ability of the formaldehyde vapors to penetrate covered surfaces.
  - The fumes of formaldehyde are noxious; they are irritating to tissues and eyes.

- Mode of Action.
  - Formaldehyde is an extremely reactive chemical.
  - It combines readily with vital organic nitrogen compounds such as proteins and nucleic acids → interaction with these cellular substances accounts for its antimicrobial action

2.6 Gaseous agents

- For heat labile substances
  - Plastic syringes, blood transfusion apparatus, and catheterization equipment
  - Plastic pipettes, petri dishes, and other equipment, that is packaged and sterilized ready for use.
  - To disinfect or sterilize an enclosed area
- The main agents currently used for gaseous sterilization
  - Ethylene oxide
  - β-propiolactone
  - Formaldehyde

ETHYLENE OXIDE

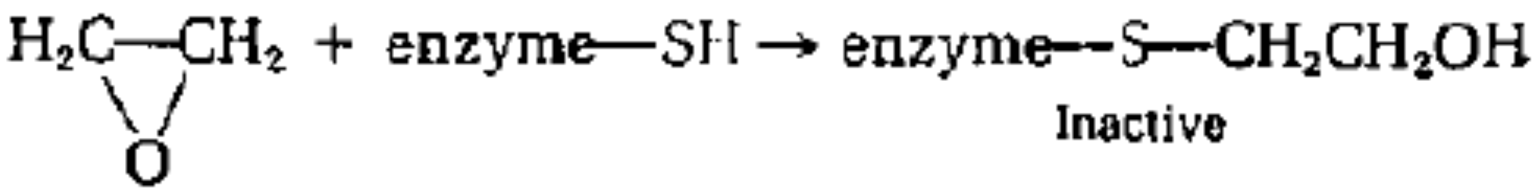


- Liquid at temperatures below 10.8°C
- Above this temperature, it vaporizes rapidly.
- Vapors in air are highly flammable even in low concentrations.
  - This overcome by preparing mixtures in carbon dioxide or Freon
    - The carbon dioxide–ethylene oxide or Freon–ethylene oxide mixtures are nonflammable,
    - there is no alteration of the microbicidal activity of the ethylene oxide.
    - The carbon dioxide and the Freon merely serve as inert diluents to prevent flammability.
- Powerful sterilizing agent
  - Heat- or moisture-sensitive materials in hospitals, industry, and laboratories
    - Spices, biological preparations, soil, plastics, certain medical preparations, and contaminated laboratory equipment.
    - Americans and the Russians for decontaminating spacecraft components.
  - Bacterial spores show little resistance to destruction by this agent.

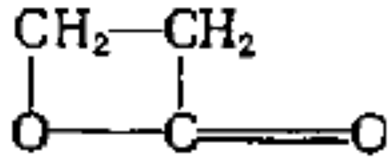
- Power to penetrate
  - It will pass through and sterilize large packages of materials, bundles of cloth, and even certain plastics
- The commercially available apparatus for this purpose is essentially an autoclave modified to allow the chamber to be filled with the gas under controlled conditions.
- The concentration of ethylene oxide, as well as the temperature and humidity, are critical factors which together determine the time required to achieve sterilization.
- Among the advantages : remarkable penetration and its broad spectrum of activity against microorganisms, including spores; effective at relatively low temperatures, and it does not damage materials exposed to it.
- One of its disadvantages is its comparatively slow action upon microorganisms.

Mode of Action.

- Alkylation reactions with organic compounds such as enzymes and other proteins.
  - Alkylation consists in the replacement of an active hydrogen atom in an organic compound, e.g., the hydrogen atom in a free carboxyl, amino, or sulfhydryl group, with an alkyl group.
  - In this reaction the ring in the ethylene oxide molecule splits and attaches itself where the hydrogen was originally.
  - This reaction would inactivate an enzyme with a sulfhydryl group:



β-PROPIOLACTONE



- Colorless liquid at room temperature with a high boiling point (155°C)
- Highly soluble in water; miscible with ethanol, acetone, diethylether, chloroform
- Considerably more effective in destroying microorganisms on surfaces
  - sporicidal, fungicidal, and virucidal
- Not flammable like ethylene oxide; lacks the penetrating power of ethylene oxide
- Vesicant and lachrymator → must be handled with care
- Whereas the usual concentration of ethylene oxide for sterilization purposes is 400 to 800 mg/liter, only 2 to 5 mg of β-propiolactone is required.
- However, the fact that it has a low power of penetration coupled with its alleged carcinogenic properties has restricted its use as a practical sterilizing agent.

2.7 Dyes

<u>ACRIDINE DYES</u>

- Acridine derivatives : acriflavine and tryptoflavine.
  - Exhibit selective inhibition against bacteria, particularly staphylococci and gonococci.
  - Gonococci are inhibited by tryptoflavine in dilutions of 1:10,000,000 to 1:50,000,000.
  - They possess little, if any, antifungal activity.
- Presently, they have less application due to advent of antibiotics
- They are used to some extent for the treatment of burns and wounds and for ophthalmic application and bladder irrigation.

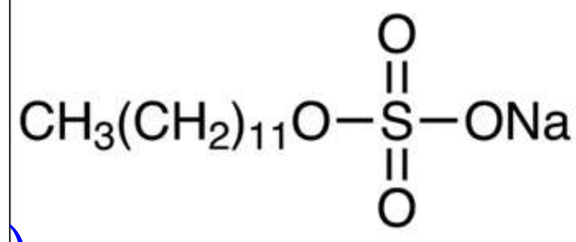
TRIPHENYLMETHANE DYES

- Malachite green, brilliant green, and crystal violet
- GP are more susceptible to lower concentrations than GN.
  - Crystal violet inhibit GP cocci at a dilution of 1:200,000 to 1:300,000; 10 times this concentration is required to inhibit *E. coli*.
  - *S. aureus* is inhibited by malachite green at a concentration of 1:1,000,000; a concentration of about 1:30,000 is required to inhibit *E. coli*.
- Selective media → incorporation of low conc (about 1:100,000) of the dyes.
  - GP bacteria will be inhibited; Media of this kind are used extensively in public health microbiology, where detection of *E. coli* is important.
- Crystal violet has also been used as a fungicide.
  - A concentration of 1:10,000 is lethal for *Monilia* and *Torula*, and a concentration of 1:1,000,000 is inhibitory.
- Mode of Action.
  - Uncertain, but there is speculation that they exert their inhibitory effect by interfering with cellular oxidation processes.

2.8 Synthetic Detergents

- Cleansing surfaces
- Soap is a poor detergent in hard water.
  - Many new more efficient cleaning agents developed, called **surfactants or synthetic detergents**
    - Superior to soap.
    - They do not form precipitates in alkaline or acid water, nor do they produce deposits with minerals found in hard water.
- Extensively used in laundry and dishwashing powders, shampoos, and other washing preparations.
- Some are also highly bactericidal.

- Categories
  - Anionic detergents (detergent property resides in the anion)
    - For example, sodium lauryl sulfate, soap

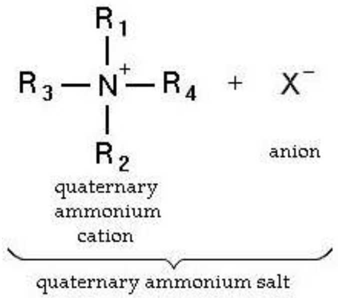


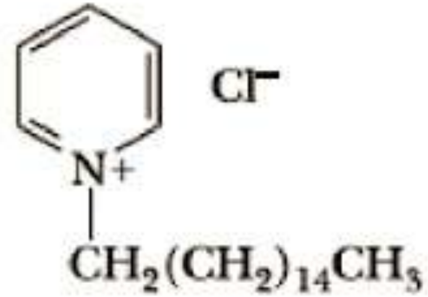
  - Cationic detergents.
    - For example, quaternary ammonium salts (ceepryn)
    - Cationic detergents are regarded as more germicidal than anionic compounds
  - Nonionic detergents; i.e.. they do not ionize; not significant antimicrobial activity.

<u>Mode of Action</u>

- Soaps reduce surface tension, and thereby increase the wetting power of the water.
- Soapy water has the ability to emulsify and disperse oils and dirt.
- The microorganisms become enmeshed in the soap lather and are removed by the rinse water.

2.9 Quaternary Ammonium Salts





Cetylpyridinium chloride

- Most germicidal cationic-detergent class are quaternary ammonium salts.
- The R groups may be any one of a large number of different alkyl groups.
  - E.g. ceepryn, cetrimide, zephiran
- The bactericidal power of the quaternaries is exceptionally high against GP bacteria, and they are also quite active against GN organisms.
- Bactericidal concentrations range from dilutions of one part in a few thousand to one part in several hundred thousand; ability to manifest bacteriostatic action at very low concentration.
  - For example, the limit of bactericidal action for a given compound may be at a dilution of 1:30,000; yet it may be bacteriostatic in dilutions as high as 1:200,000.

- Fungicidal + destructive to certain of the pathogenic protozoa. Viruses appear to be more resistant than bacteria and fungi.
- Germicidal activity, detergent action, low toxicity, high solubility in water, stability in solution, and noncorrosiveness
  - Disinfectants and sanitizing agents.
    - Floors, walls, and other surfaces in hospitals, nursing homes, and other public places
    - They are used to sanitize food and beverage utensils in restaurants as well as surfaces and certain equipment in food-processing plants.
    - Other applications are to be found in the dairy, egg, and fishing industries to control microbial growth on surfaces of equipment and the environment in general
  - They are used as skin disinfectants, as a preservative in ophthalmic solutions, and in cosmetic preparations.

Mode of action.

- Denaturation of proteins, interference with glycolysis, and membrane damage.
- Damage to the cell is the cytoplasmic membrane → alter the vital permeability features of this cell structure.

Table 23-8. Sites of Action of Antimicrobial Chemical Agents Other Than Antibiotics

	Chemical Agents										
Sites of Action*	Acridine Dyes	Alcohols	Chlorine and Chlorine Compounds	Ethylene Oxide	Formaldehyde	Glutaraldehyde	Heavy-metal Salts	Iodine	Phenols	β-Propiolactone	Quaternary Compounds
Cell wall									+		
Cytoplasmic membrane		+							+		+
Proteins (denaturation)		+					+		+		+
Nucleic acids	+					+					
Enzymes with sulfhydryl (SH) groups			+	+		+	+	+		+	
Amino acids			+	+	+	+				+	

* In some instances the site of action is dependent upon the concentration of the chemical.

Chemical Agent	Recommended Use	Limitations
Phenol and phenolic compounds	General disinfectant	Microbial effectiveness limited; irritating and corrosive
Alcohols: ethyl and isopropyl	Skin and thermometer antiseptic	Antiseptic
Iodines	Disinfect skin	Irritating to mucous membranes
Chlorine	Water disinfection	Inactivated by organic material; pH dependent for effectiveness; objectionable taste and odor unless strictly controlled
Silver nitrate	Treating burns	Possibie irritation
Mercurials	Skin disinfection	Slow-acting; toxic
Quaternaries	Skin disinfection	Not sporicidal
Formaldehyde	Sterilizing instruments; fumigation	Permeation poor; corrosive
Glutaraldehyde	Sterilizing instruments; fumigation	Stability limited
Ethylene oxide	Sterilizing heat-sensitive materials, instruments, and large equipment	Flammable; potentially explosive in pure form
β-propiolactone	Sterilizing instruments and heat-sensitive materials	Lacks penetrating power

Properties of an Ideal Disinfectant

- Antimicrobial activity
  Broad-spectrum
  Low concentration
- Toxicity to microbes at room temperature
- Quick action against MOs, not cause stasis leading to false sense of security
- As specific as possible for MOs and not inactivated by extraneous material
- Non-toxic to humans and other animals
- Homogenous and uniform preparation so that active ingredients are present in each application
- Easy to apply, easy to handle
- Solubility & stability
- Good surface tension reducer, good wetting agent, penetrating power
- Cheap, readily available, non-staining, non-corroding, pleasant smell

Evaluation of Disinfectants

- No single disinfectant has all ideal properties
- Effectiveness as microbicidal agent is checked by mixing the microbes and disinfectant – conditions need to be standardized
  - Concentration of disinfectant
  - Time of incubation
- Test microorganism
  - Each the chemical agent is tested against a specified microorganism referred to as the test organism

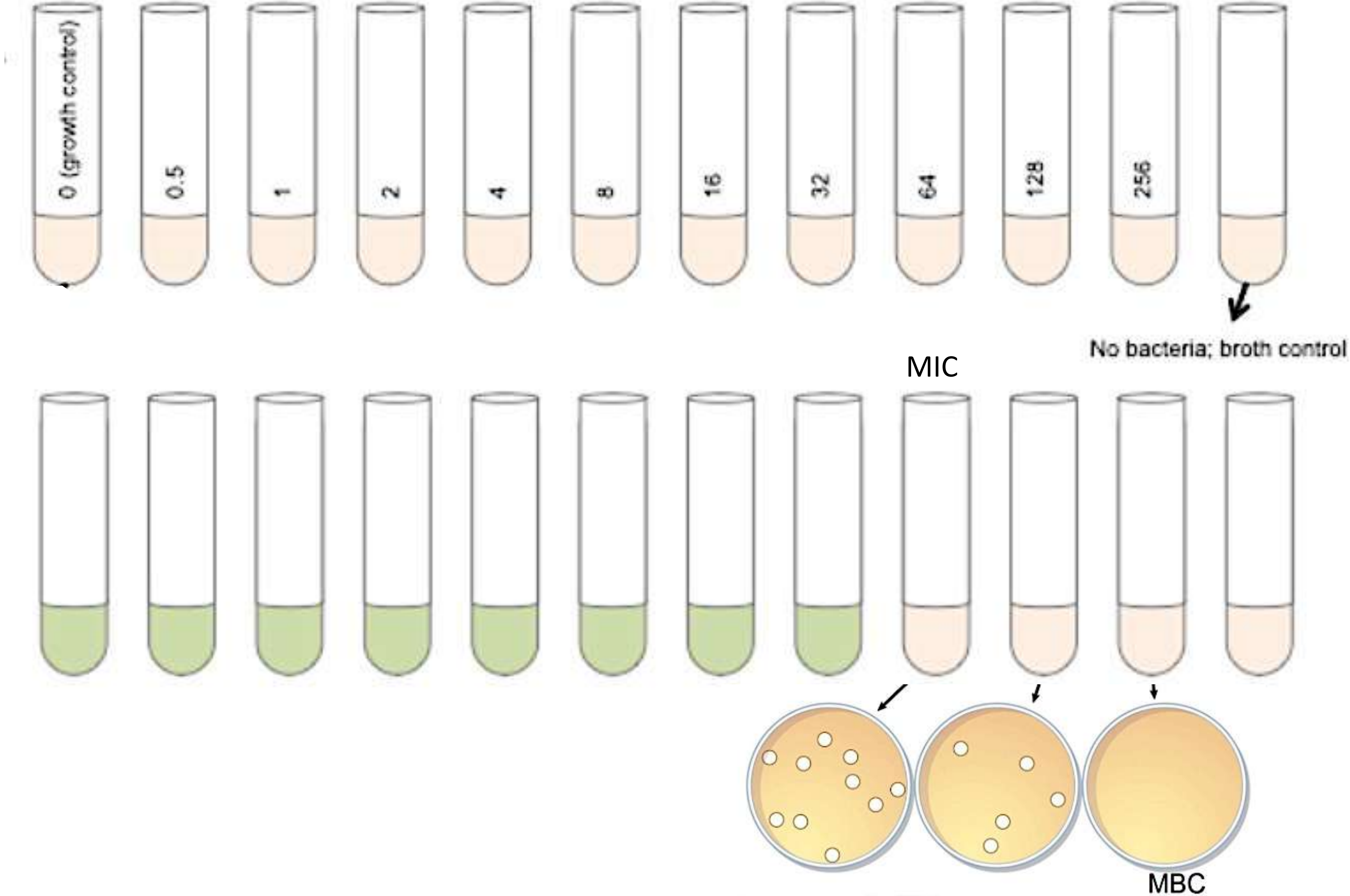
1. Tube-dilution method

Series of dilution of disinfectant in water / nutrient broth

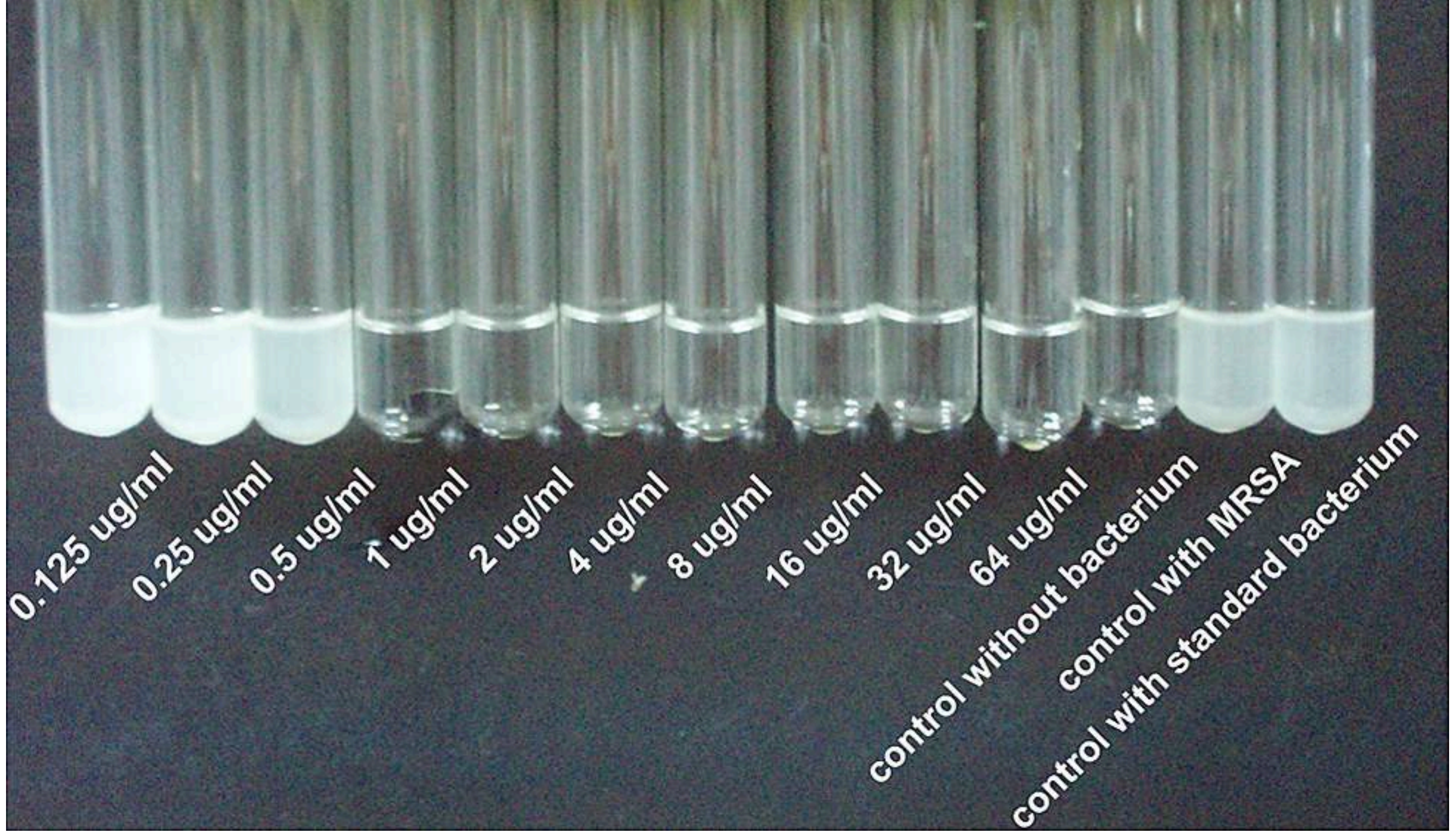
Add measured amount of test microorganism → Incubate → observe for growth

Minimum inhibitory concentration (MIC) – observe turbidity

Minimum bactericidal concentration (MBC) - Take out aliquot → add to media → observe for growth



0 (growth control)
0.5
1
2
4
8
16
32
64
128
256
No bacteria; broth control
MIC
MBC


0.125 ug/ml
0.25 ug/ml
0.5 ug/ml
1 ug/ml
2 ug/ml
4 ug/ml
8 ug/ml
16 ug/ml
32 ug/ml
64 ug/ml
control without bacterium
control with MRSA
control with standard bacterium

2. Agar plate techniques

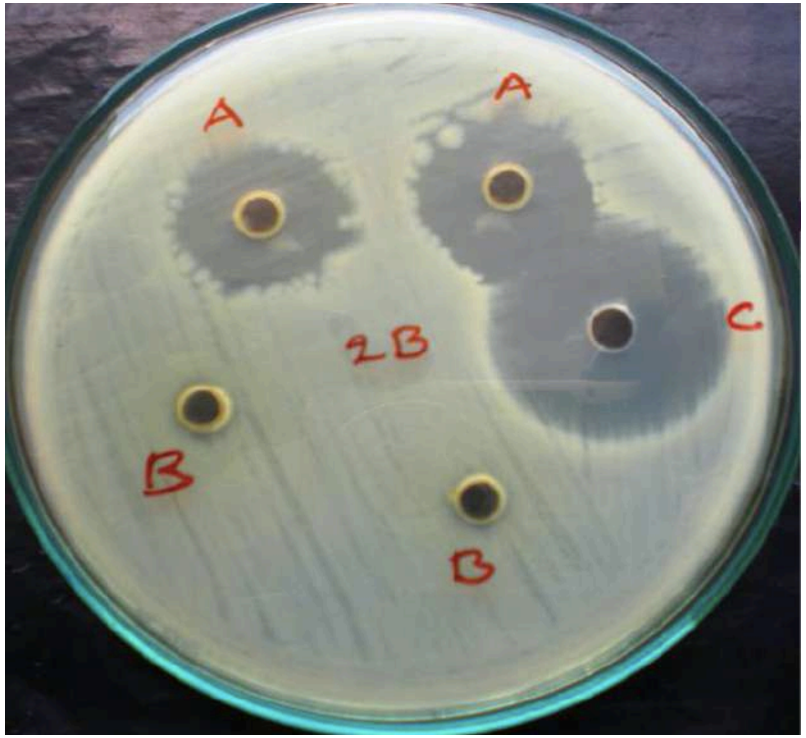
Test microorganism swabbed onto agar plate (spread plate)

↓

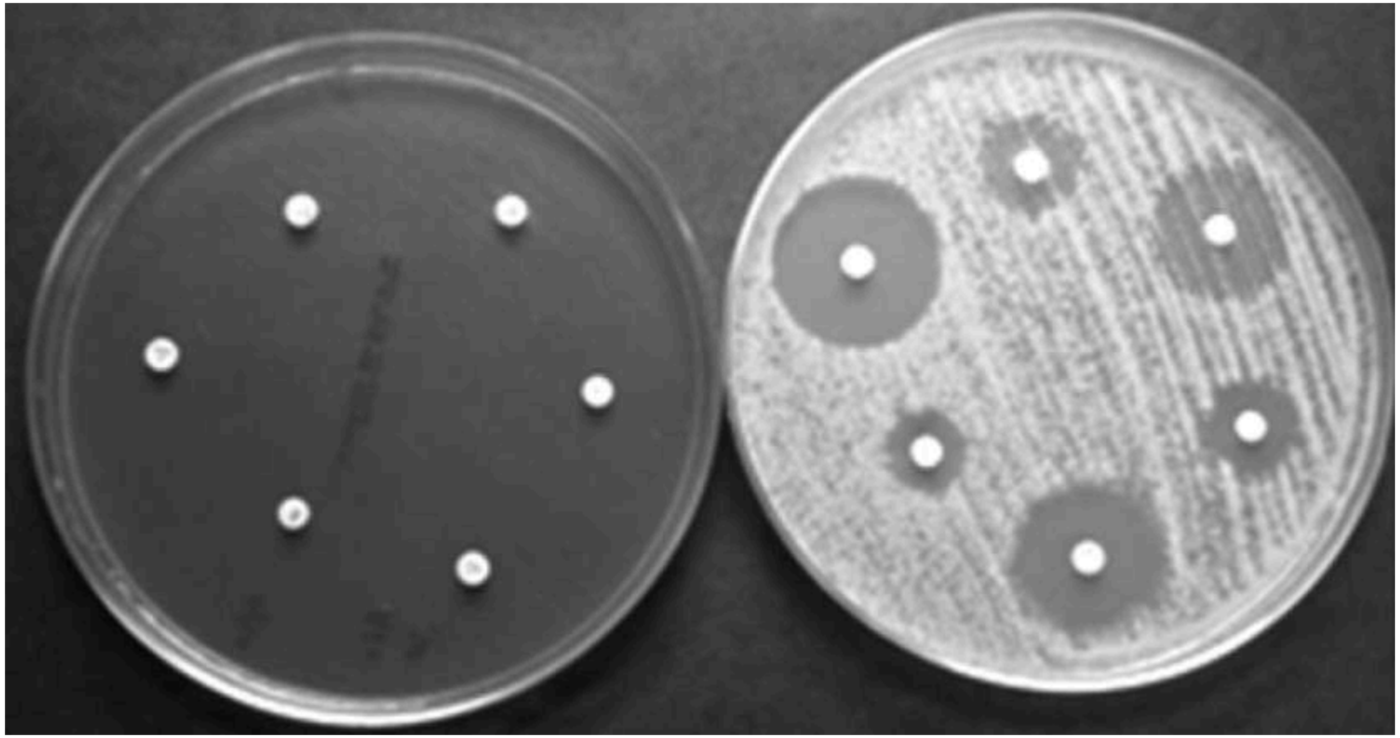
Disinfectant placed on medium loaded onto discs / added into the wells

↓

Incubate overnight → Observe zone of inhibition



Well-diffusion technique



Disc-diffusion technique

3. Phenol Coefficient Method

- A specific official test (FDA & AOAC)
- Suitable for testing disinfectants miscible with water and exerting their antimicrobial action in a manner similar to that of phenol.
- The test organism : specific strain of either *Salmonella typhi* or *Staphylococcus aureus*.
  - The temperature at which the test is performed, the manner of making subcultures, the composition of the subculture medium, the size of the test tubes, and other details of the test are spelled out in the official procedure.

To series of dilutions of disinfectant being tested (5ml), 0.5ml of 24-h culture of the test organism is added.

↓

Similar additions, in the same amounts, are made to a series of dilutions of phenol, at the same time

↓

All tubes (disinfectant + organisms and phenol + organisms) are incubated at 20°C

↓

At intervals of 5, 10, and 15 min, subcultures are made with a loop into sterile media tubes.

↓

The inoculated subculture tubes are incubated and subsequently examined for growth

- **Phenol coefficient** of the substance tested is the ratio of :

$$\frac{\text{The highest dilution of } \textbf{disinfectant} \text{ killing the test organism in 10 min but not in 5 min}}{\text{The highest dilution of } \textbf{phenol} \text{ showing the same result}}$$

		Subculture Tubes*		
	Dilution	5 min	10 min	15 min
Disinfectant (X)	1:100	0	0	0
	1:125	+	0	0
	1:150	+	0	0
	1:175	+	+	0
	1:200	+	+	+
Phenol	1:90	+	0	0
	1:100	+	+	+

Phenol coefficient of (X) = $150/90$ = 1.6

More than 1 (more effective tested disinfectant compared to phenol); Less than 1 (less effective); Equal to 1 (equally effective)

Thank you…