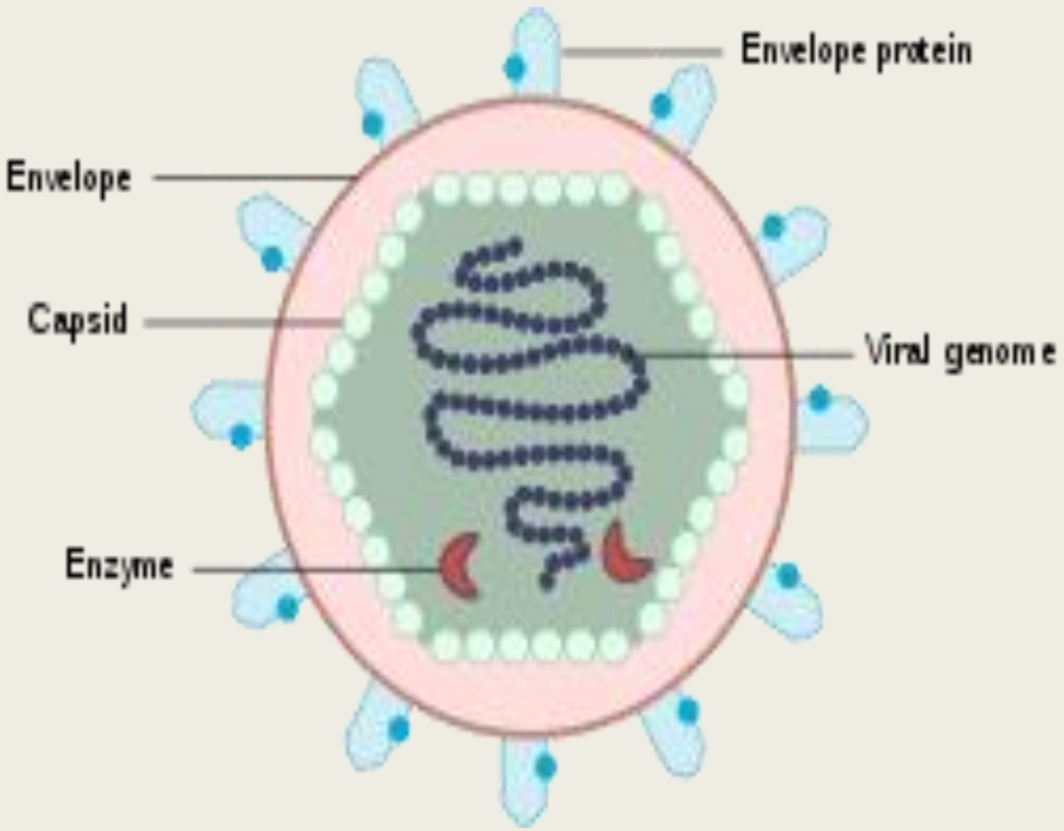
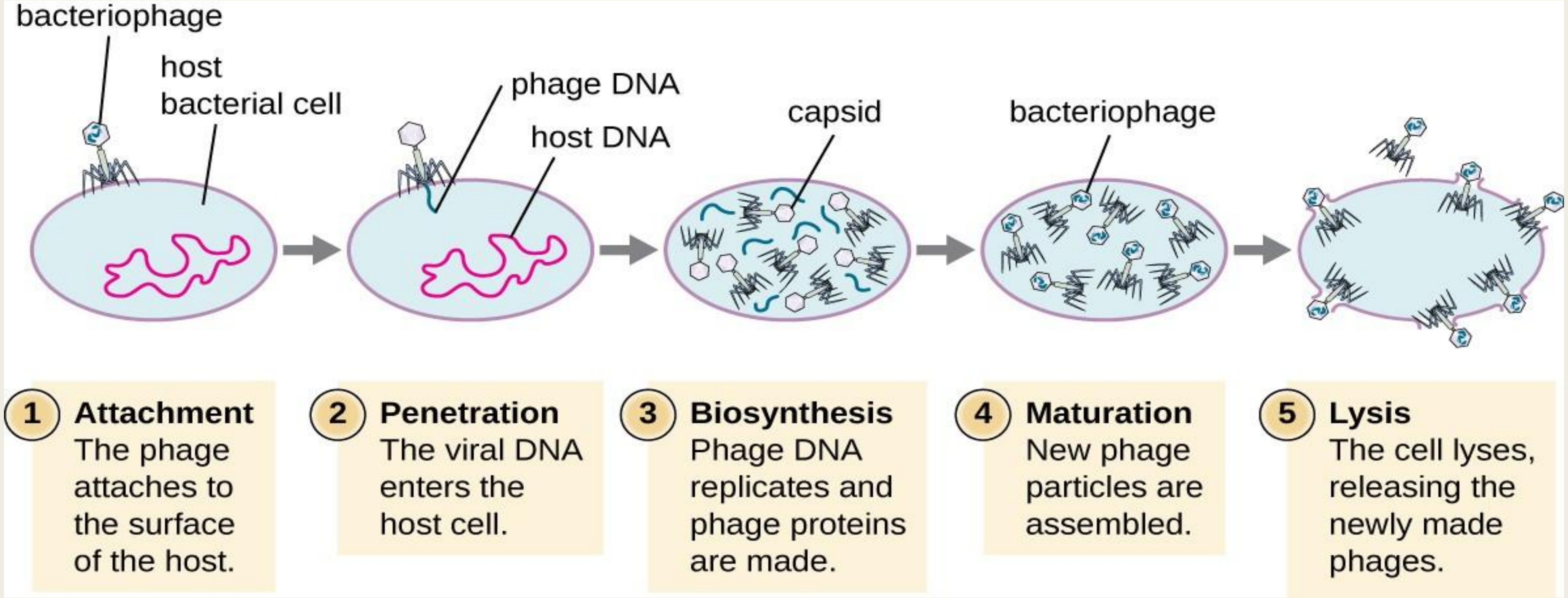
Antiviral Drugs

Viruses

- A virus is a small infectious agent that replicates only inside the living cells of an organism.
- A strand of genetic material either DNA or RNA.
- A protein coat capsid.
- Viruses do not have ribosomes, mitochondria or other cell organelles.
- They cannot reproduce outside a host cell.
- They are obligate parasites



Viral Replication



All steps of replication can be a target for antiviral drugs.

- Virion destruction
- Attachment
- Penetration and peeling
- RNA synthesis
- Replication
- Protein synthesis
- Virion maturation and release stages

- Unlike antibacterial drugs, the effect of many antiviral drugs is limited to a particular virus.
- The high rate of mutations in viruses is problematic for the development of resistance to antivirals.

Virion destruction

- Enveloped viruses are susceptible to many lipid-solvent and detergent-like molecules, since they break down the envelope membrane to prevent the virus from adhering to the cell.
- For example; Nonoxinol-9 : A detergent-like substance can inactivate Herpes Simplex Virus (HSV) and Human Immunodeficiency Virus (HIV)

Attachment

- This interaction with **neutralizing anti**bodies and **receptor antagonists (peptide analogues**) can be prevented.

Penetration and peeling

Penetration and peeling are the necessary steps for transferring the viral genome to the host cytoplasm.

- Plekonaril and other methyl-isoxazole compounds: prevents the separation of capsid.
- Amantadine, rimantidine and other hydrophobic amines: Prevents the peeling of the virion by neutralizing the pH of the medium
- Tromantidine: Prevents penetration and peeling.

RNA synthesis

Although mRNA synthesis is responsible for the proliferation of viruses, this molecule is not a good target for antivirals. Because it is difficult to inhibit viral mRNA synthesis without allowing cellular mRNA synthesis.

- Guanidine and 2-hydroxybenzylbenzimidine, ribavirin, isatin-β-thiosemicarbazone are involved as antivirals that inhibit RNA synthesis.

Protein synthesis

- Viral protein synthesis is a weak target for antivirals. Since viruses use the cell's ribosomes and synthesis mechanisms for replication, selective inhibition is not possible
- Interferon-α and Interferon-β inhibit viral protein synthesis of infected cells.

Genom replication

Most antivirals; **nucleoside analogs** which vary in base, sugar or both. These analogs are different from host enzymes such as DNA polymerase and reverse transcriptase enzymes.

- Acyclovir, ganciclovir, ribavirin, 5-iododeoxyuridine (idoxuridine), trifluorothymidine, phosphonoformic acid, nevirapine, delavirdine.

Viral maturation and release

- HIV protease is a unique and essential enzyme for virion maturation and production of infectious virions.

Sacquinavir, ritonavir, indinavir: protease inhibitors

- Neuraminidase of influenza virus is also an antiviral target.

Zanamivir (Relenza) and oseltamivir (Tamiflu)

Nucleoside analogs

Most antiviral drugs approved by the US Food and Drug Administration (FDA) are nucleoside analogs that inhibit viral polymerase. The development of resistance to these drugs is caused by mutations in the polymerase.

- Acyclovir, valaciclovir, penciclovir and famciclovir
- Gabciclovir, valganciclovir
- Sidofovir and adefovir
- Azidothymidine, Dideoxyuridine, dideoxycytidine, stavudine and lamivudine
- RibavirinIdoxyuridine, trifluorothymidine, fluorouracil

Non-nucleoside analogs

- Foscarnet
- Nevirapine, delavirdine, efavirenz

CLASSIFICATION OF ANTIVIRAL DRUGS

The viral growth cycle	Selective inhibitors
1) Attachment ***2) Penetration***	-Antiviral antibodies (gamma globulin)
3) Uncoating	**-Amantadine**, rimantadine -Interferons
4) Early translation (early mRNA and protein synthesis)	fomivirsen
5) Transcription (viral genome replication)	***Inhibitors of DNA-polymerase*** **-Acyclovir** **-Gancyclovir** -Famcyclovir -Cidofovir **-Vidarabine** -Idoxuridine -Trifluridine **-Foscarnet** ***Inhibitors of RNA-dependent DNA-polymerase (reverse transcriptase)*** **-Zidovudine** -Didanosine -Stavudine -Zalcitabine -Lamivudine -Foscarnet

Classification of antiviral drugs according to their therapeutic uses

- **Anti-herpes virus agents**

  Acyclovir, Famcyclovir, Gancyclovir, Idoxuridine, Foscarnet, Fomivirsen, Pencyclovir, Trifluridine, Tromantadine, Valacyclovir, Valgancyclovir, Vidarabine, Cidofovir, Docosanol

- **Anti-influenza Agents**

  Amantadine, Oseltamivir, Peramivir, Rimantadine, Zanamivir

- **Other antiviral agents**

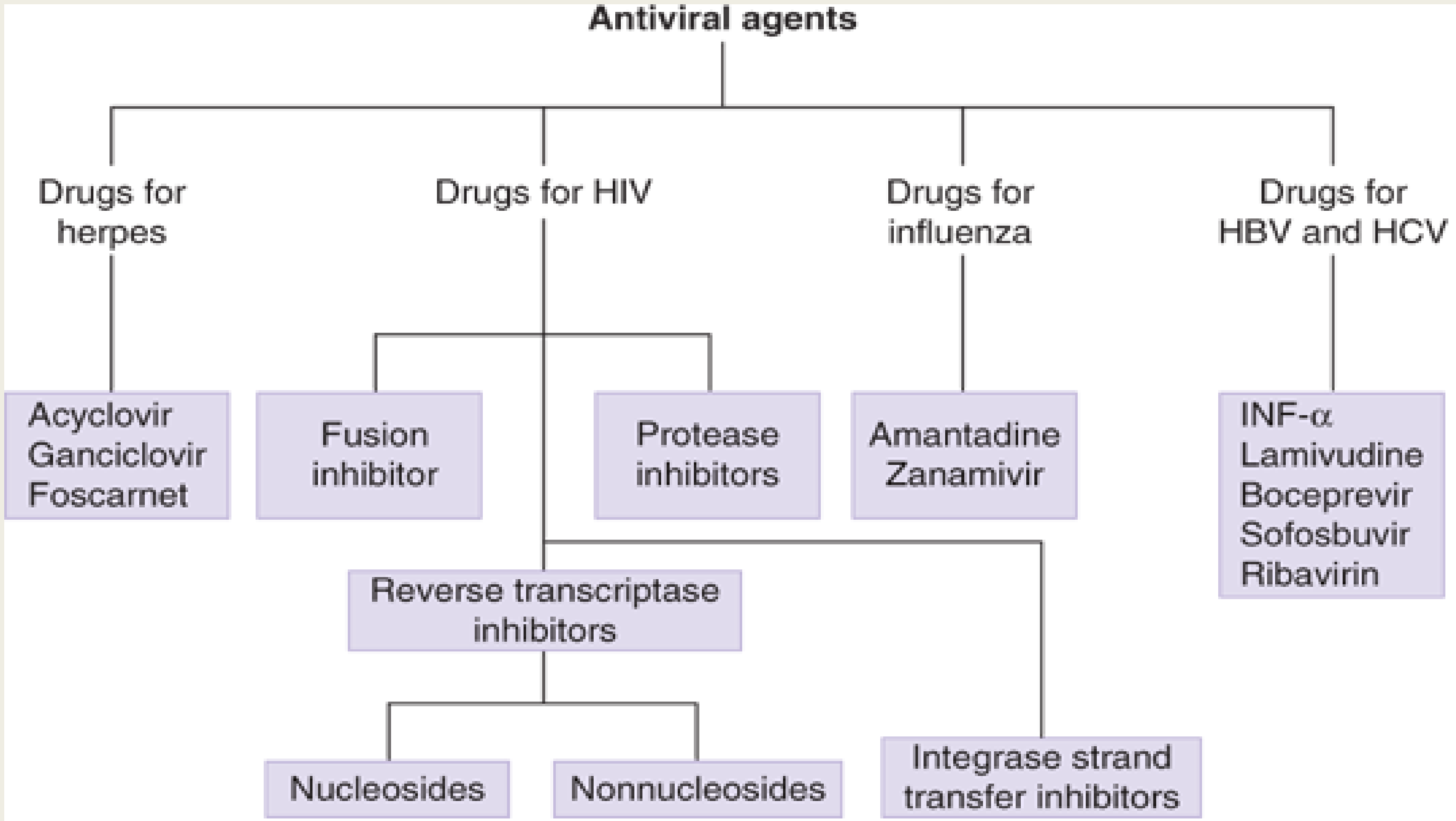
  Fomivirsen, Enfuvirtide, Imiquimod, Interferon, Ribavirin, Viramidine

- **Antiretroviral Agents**

➢ NRTIs: Zidovudine, Didanosine, Stavudine, Zalcitabine, Lamivudine, Abacavir, Tenofovir

➢ NNRTIs: Nevirapine, Efavirenz, Delavirdine

➢ Protease Inhibitors: Saquinavir, Indinavir, Atazanavir, Ritonavir, Nelfinavir, Amprenavir, Lopinavir, Tipranavir



Source: A.J. Trevor, B.G. Katzung, M. Kruidering-Hall: Katzung & Trevor's Pharmacology: Examination & Board Review, 11th Ed.
www.accesspharmacy.com

Antifungal Drugs

Antifungal Drug Class	Drug	Mode of Action
Azoles	Fluconazole Voriconazole Posaconazole Itraconazole Ketoconazole Clotrimazole Econazole	Inhibitor of lanosterol 14α—demethylase
	Miconazole	
Echinocandins	Caspofungin Anidulafungin	Inhibitor of 1,3–β–glucan synthase
	Micafungin	
Polyenes	Amphotericin B	Binding to ergosterol
	Nystatin	
Pyrimidine analogue	flucytosine	Inhibitor of DNA/RNA/protein synthesis

- **Azoles:** Inhibits cytochrome P450 enzyme

Inhibits the synthesis of ergosterol, a sterol specific to the fungal membrane

- **Echinocandins:** Inhibits beta (1-2) glycan synthesis in the fungal cell wall.
- **Polyenes:** binding to ergosterol
- **Pyrimidine analogue**: Inhibit of DNA/RNA/ protein synthesis

Antiparasitic Drugs

Antiparasitics, drugs which kill or inhibit the growth of parasitic organisms, may be subdivided into the following major therapeutic categories:

- Antiprotozoal
- Antihelminthic
- Antifilarial
- Antimalarial

Antiprotozoal drugs

- The mechanisms of antiprotozoal drugs differ significantly drug to drug.
- **Eflornithine**, a drug used for treating trypanosomiasis, inhibits ornithine decarboxylase.
- Aminoglycoside antibiotic/antiprotozoal used to treat leishmaniasis are thought to inhibit protein synthesis.

Mechanisms of Action & Clinical Indications for the Major Anti-parasitic Agents – *Anti-protozoal Agents*

Drug Class	Mechanism of Action	Examples	Clinical Indications
Heavy metals: arsenical and antimonials	• Inactivate sulfhydryl groups. • Disrupt glycolysis.	Melarsoprol, sodium stibogluconate, meglumine antimonate	Trypanosomiasis, Leishmaniasis
Aminoquinoline analogues	• Accumulate in parasitized cells. • Interfere with DNA replication. • Bind to ferriprotoporphyrin IX. • Raise intravesicular pH. • Interfere with hemoglobin digestion	Chloroquine, mefloquine, quinine, primaquine, halofantrine, lumafantrine	Malaria prophylaxis and therapy Radical cure (exoerythrocytic- primaquine only)
Folic acid antagonists	• Inhibit dihydropteroate synthetase and dihydrofolate reductase	Sulfonamides, pyrimethamine, trimethoprim	Toxoplasmosis, malaria, cyclosporiasis
Inhibitors of protein synthesis	• Block peptide synthesis at levelof ribosome	Clindamycin, spiramycin, paromomycin, tetracycline, doxycycline	Malaria, babesiosis, amebiasis, cryptosporidiosis, leishmaniasis, onchocerciasis

Antihelmintics

- These are the substances used to inhibit or kill helminthic/worm parasites. These are also called wormers. Helminths are parasitic worms that feed on a living host to gain nourishment and protection, while causing poor nutrient absorption, weakness and disease in the host. It can be antinematodal, anticestodal as well as antitrematodal.

- **Antinematodal:** Substances used in the treatment of nematode infection i.e. roundworm infections. Some examples are piperazine, imidazothiazoles, benzimidazole a tetrahydropyrimidines.
- **Anticestodal:** Drug used to combat tape worm infection It include natural organic compound arecoline and the synthetic compounds bunamidine, dichlorophen, praziquantel, uredofos, niclosamide and resorantel etc.
- **Antitrematodal:** It Includes albendazole, bithionol sulfoxide, bromsalans, carbon tetrachloride,clioxanide, niclofolan, triclabendazole etc.

Antimalarials

They are drugs mainly used to control symptoms of malaria, e.g. chloroquine & primaquine.

- **Chloroquine** oral tablet plays a role in the invasion of red blood cells within the parasite period, which will effectively control the onset of symptom.
- **Primaquine** is used for the prevention of malaria recurrence and spread of drugs. It is mainly used for eradicating vivax malaria and controlling malaria transmission.

Antifilarials

- These are agents to control filariasis , parasitic disease caused by an infection with roundworms of the Filarioidea type. Ivermectin, diethylcarbamazine etc are commonly used antifilarials.

EFFECT OF PHYSICAL FACTORS ON MICROORGANISMS

STERILIZATION-DISINFECTION

Assoc. Prof. Banu KAŞKATEPE

OBJECTIVES

- To explain sterilization, disinfection and antisepsis concepts
- The methods of sterilization
- To know the disinfectant usage areas
- To learn the mechanism of action of disinfectants

1. pH

- H ion concentration.
- *Although the pH requirements of the m.o are different, they usually grow good at pH: 6-8. According to pH requests m.o are divided into 3 categories*
  - Acidophilic: 1-5
  - Neutrophilic: 6-8
  - Basophilic: 8.5-12
- *Bacteria are generally neutrophilic, most of the fungi have acidophilic character.*

2. Oxygen

- Aerobic microorganisms
- Anaerobic microorganisms
  - Compulsory anaerobe
  - Facultative anaerobe
  - Microaerophils (2-10% O_2 requirement)
  - Aerotolerant: It reproduces in the presence of O_2 but does not use O_2.

3.Heat

For each species of microorganism, depending on the enzyme work, there is a minimum , a maximum , and an optimal temperature limit that reproduction can be best.

Microorganisms according to optimum reproduction rates; psychrophiles, mesophiles and thermophiles

	reproduction	dead
■ Psychrophiles bacteria=>	-8 - +15 °C	30 - 35 °C
■ Mesophiles bacteria=>	20 - 45 °C	70 °C
■ Thermophiles bacteria=>	50 -70 °C	100 - 110 °C

Thermal time of death => Time required for a known microorganism to die at a certain temperature.

Thermal point of death => The rate of heat that kills a known microorganism within a certain period of time.

D value (mortality rate): time required to reduce one logarithmic unit (90% reduction) of the number of microorganisms present. It is related to the number of microorganisms at the beginning. D_{105}= 2 min

A- High temperature: It has effect on reproduction and character change.

- *Bacillus anthracis* loses its ability to form sports at 42°C with a few passages.

Heat resistance grade of bacteria depends on various factor;

- During the period of the effect,
- The genus of the bacteria
- The reproduction period,
- various factors in the environment. (the numbers of bacteria, the composition of environment, the pH of env., humidity etc.)

- B- Low temperature: (Cold): Microorganisms are highly resistant to cold and extreme cold. Some are able to withstand even at -80/-190 °C. With cold effect, cell metabolism slows down and stops, It can not perform its vital functions and can not reproduce. In extreme cold application, the time required to reach the desired low temperature is also important. The situation is different for suddenly cooled to -10ºC or slowly cooled.

4. Dryness

Resistance to dryness is depends on

a- the type of microorganism

b- the biological situation

Water is important for the bacteria because they need water for their biochemical reactions, taking nutritional compound (the compound solve in the water), pumping toxins. In dryness m.o loses the water, a lot of biochemical reactions stop and bacteria die.

5. Pressure

- **1- High pressure:** Microorganisms are basically resistant. After prolonged application (10,000 atmospheres) the resulting of the protein denaturates, microorganisms die but they do not break down. With Sudden changes, 500-600 atmospheres pressure is repeatedly applied several times, microorganisms are broken down and die.
- **2- Crushing press**

- **3- Osmotic pressure.** Bacteria maintain their liveliness between 0.5% and 3% NaCl pressure limits by controlling intracellular osmosis and ion density.
- If environmental osmotic pressure ↑ m.o lose water and membrane of bacterium contracted: plasmolysis. If environmental osmotic pressure decrease, more water come into the bacteria cell from environment and bacteria burst : plasmoptysis.

6. Sonic and ultrasonic vibrations

- 100 - 10,000 vibrations / second = sonic
- 30,000 - 140,000 vibrations per second = ultrasonic

With the effect of both vibrations, the cells break down, the enzymatic functions stop, and the proteins become coagulated.

7. Rays

Ultraviolet rays = 200 - 280 nm. 2537 A ° is used. (Non-ionized)

- Form thymine and cytosine dimers.
- Prevents synthesis of tyrosine, cystine and tryptophan
- It also indirectly influences with the formation of ozone and hydrogen peroxides.

Ionized rays = (Beta, gamma and X rays)

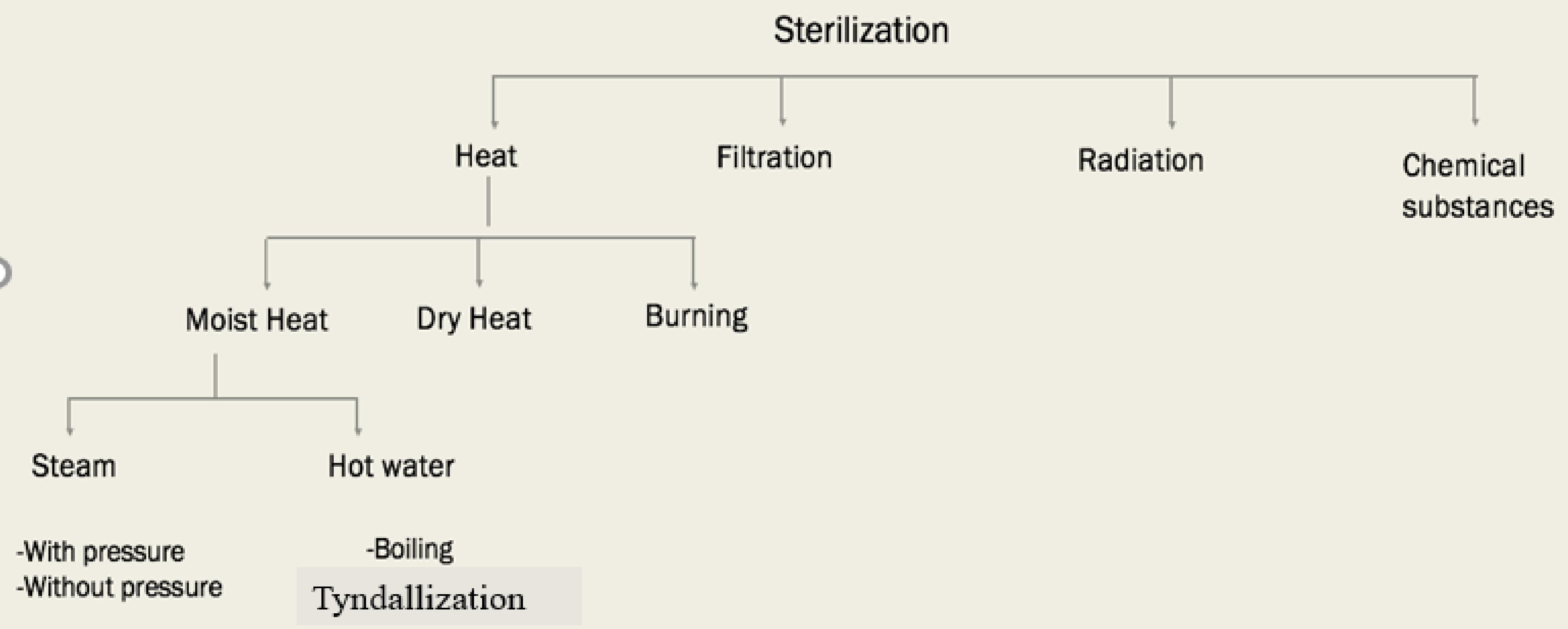
- * ionizes water , -OH and + H They are in the ability to penetrate and ionize.
- They are used in the sterilization of medical materials and certain foods.

STERILIZATION

DISENFECTION

ANTISEPSIS

- Sterilization: Destruction of all forms of microbial life
- Disinfection: Chemical destruction of vegetative pathogens inanimate environment
- Antisepsis: Chemical destruction of vegetative pathogens on living tissue



Sterilization
Heat
Filtration
Radiation
Chemical substances
Moist Heat
Dry Heat
Burning
Steam
Hot water
-With pressure
-Without pressure
-Boiling
Tyndallization

Heat usage in Sterilization

- **Dry heat kills bacteria by oxidative pathways, mouist heat kills bacteria by coagulating proteins.**
- When the proteins are heated in a humid environment, separated into smaller peptide chains and the SH groups are cleaved .
- In the absence of water, the polar groups in the peptide chains are less active.
- The difference between dry and moust heat is transmitting of heat energy. Water vapor transmit the heat energy better.

- Heat sterilization is the

- The least toxic
- Safest
- Most economical
- Easily applicable

sterilization method.

Sterilization with steam under pressure

- Autoclave: It is used especially in the sterilization of media and materials which can withstand 121 ° C. It is used under pressure. Generally we use 121 ° C under 1 atm pressure for 15 -20 minutes. 134 ° C under 2 atm for 3-4 minutes
- CDC recomends this methods

Sterilization with steam (without pressure)

(Koch steam sterilizer): 100 ^{o}C for 1 hour, sugar solutions can not withstand high temperatures.

- Tyndallization: Intermittent sterilization by exposure to steam at 100^{o}C 30 min then 85 ^{o}C 1 min. for three successive days.
- Used for sterilization of sugar media which decompose at high temperatures.
- The principle is that one exposure will kill only vegetative bacteria. Between heatings, the spores will vegetate to be killed during subsequent exposure.

Filtration
The Types of Filters

1. Berkefeld filter: from diatomaceous soil

2. Pasteur ve Chamberland filter

3. Seitz filter from Compressed asbestos

4. Filters made of compressed glass powder

5. **Membrane filters.** It is made from collodion (cellulose nitrate or cellulose acetate).

0.005- 1 micron . Makes mechanical filtration.

- 0.22 – 0.45 micron = bacteria holding filters
- 0.01 micron = Filters that keep even small viruses

Sterilization with chemical substances

1- **Chemical sterilant** (ethylene oxide, formaldehyde, chlorine dioxide, hydrogen peroxide gas plasma…),

2- **High-level disinfectants** (glutaraldehyde, orthophtaldehyde, peracetic acid, hydrogen peroxide…),

3- **Medium level disinfectants** (alcohols, iodine compounds, chlorine compounds, phenol compounds, chlorhexidine…) and

4- **Low-level disinfectants** (quaternary ammonium compounds…)

Sterilization with chemical substances

With Gases:

- Sterilization with ethylene oxide (C_2H_4O):
- It is liquid below 10.8 ° C. It is very toxic, irritant and explosive in pure state. Mixtures with CO_2 are used.
- Carboxide. 10% + 90%
- Oxifume. 20% + 80%

Disadvantages of ethylene oxide sterilization

- Sterilization and aeration time is long
- Liquids can not be sterilized
- Fabric (cloth) can not be used as packaging material
- can create security problems for sick, healthcare workers and environment.
- 1st grade carcinogen
- Can leave toxic residue
- It is expensive compared to steam sterilization method

Plasma Gas Sterilization (Hydrogen peroxide gas plasma)

- A reactive mixture of, electrons and excited gas molecules and free radicals formed by the energy given to the gas molecules under vacuum .

- Hydrogen peroxide (59%) is evaporated in the apparatus and reactive free radicals are formed by microwave or radio frequency energy.

- An effective method for heat and humidity sensitive materials
- Rapid sterilization (45 - 72 minutes)
- No corrosive effect,
- no toxic residue.
- Safe
- Easy to follow

- Peracetic acid = Suitable for immediate use.
- Tymol = It is used by adding to the prepared concentrates medium.
- Formaldehyde = It is is used in the sterilization of the room but restricted due to being carcinogenic
- Ozone

DISINFECTANTS AND THEIR MECHANISMS OF ACTION

- Sterilization: destruction of all forms of microbial life
- Disinfection: destruction of vegetative pathogens on inert substances
- Antisepsis: chemical destruction of vegetative pathogens on living tissue
- Disenfectants and Antiseptics are antimicrobial agents that are applied to to destroy microorganisms.
- Pasteurization: It is the process of heat processing a liquid or a food to kill pathogenic bacteria to make the food safe to eat. 72-80 $^{\circ}$C- 12-16 sc. Than 10 $^{\circ}$C



Ideal disinfectant;

- Should be fast and effective
- Should not be corrosive and non-exhaustive with cleaning agents
- should not be toxic
- should not be inactivated with organic materials
- It should be cheap
- Do not harm to the environment

Classification of Disinfectants

Disinfectants are classified according to ;

- Usage areas
- The degree of influence to microorganisms
- Chemical structure,
- The mechanisms of action.

Disinfectants according to usage areas

Instrument disinfectants

Surface disinfectants

Antiseptics

1. Instrument Disinfectants

- *Critical materials:* Materials that enter the sterile parts of the body or into the vascular system. Surgical materials, cardiac and urinary catheters, implants, ultrasound props used in sterile body cavities are critical materials. Ethylene oxide, hydrogen peroxide, glutaraldehyde
- *Semi-critical materials:* materials which come into contact with mucous membranes and deteriorated skin
- *Noncritical material:* Only materials that come into contact with intact skin.

- Disinfection should be done at a high level for critical materials, moderate for semi-critical, and low for non-critical materials. In general, high and moderate levels of disinfectants are similar. Higher disinfection times are longer than others. Ethylene oxide can only be used for high level disinfection or sterilization.
- Disinfectants such as alcohol solutions, 0.5-3% phenol solutions, iodine solutions, ammonium compounds are applied in low level disinfection process in a short time like 10 minutes.

2. Surface disinfectants

Chlorine and chlorine compounds: hypochlorite

Alcohol solutions: ethyl alcohol (70%), isopropyl alcohol

Quaternary ammonium compounds, (cationic detergent character)

Phenolics

3. Antiseptics

Soaps

Iodophores: povidone iodine, for tissue

Alcohol solutes: 50% -80% dilutions of alcohols are used.

***Pure alcohol has a weaker effect than alcohol in 70% concentration. That is why pure alcohol can not penetrate into the cell by blocking proteins on the cell wall.

Disinfectants according to their chemical structures

Inorganic compounds

Organic compounds

Inorganic Compounds

- Acids and Alkalis
- Heavy metals and salts: Copper, silver, mercury salts: These are effective at various concentrations, by coagulating proteins and disrupting enzymes. Even if it is limited, mercury-containing ointments are used in ocular infections, dermatophytes, and in the treatment of parasitic skin infections. Organic mercury compounds are used as preservatives in cosmetics and eye solutions. **1% silver nitrate solution** is instilled into the eye in order to be protected from gonococcal infection in newborns.

Oxidizing substances: Chlorine, iodine, hydrogen peroxide.

- Generally **chlorine** is used in the disinfection of drinking water and swimming pools, vegetables and fruits. In addition to chlorine, chlorine compounds such as hypochlorite and chloramines are also used. The most commonly used agent for this purpose is **sodium hypochlorite**, which is bleach. In addition, calcium hypochlorite is also used for disinfection purposes.

- **Iodine;** is an important chemical substance used in water disinfection. In addition, iodine compounds are commonly used as wound and skin antiseptics and disinfection of thermometers and surgical instruments.
- **Hydrogen peroxide** is also known as oxygenated water. It has mild antiseptic properties. It is used as a disinfectant in the disinfection of contact lenses, surgical implants, plastic instruments, and as an antiseptic in mouth and skin mouthwash.

Organic Compounds

- **Organic metal compounds**
- **Phenol and Phenolic Compounds:** both bacteriostatic and bactericidal. It acts by disrupting the semi-permeable nature of the cell membrane
- **Detergents:** Cationic detergents, Anionic detergents, Non-ionic detergents
- **Organic solvents**
- **Alkylene substances:** Formaldehyde, Gluteraldehyde, Ethylene Oxide, Betapropiolaktonpaints
- **Dyes**

- **Cationic detergents**; are chemically positively charged electrical detergents. It combines with the negatively charged parts collected on the bacterial membrane by the positive electric charge, destroys the bacteria surface and enters. In this case, the bacterium is killed by the deterioration of the semi-permeability of the bacterium. **It is effective on gram positive and gram negative bacteria**. This group includes detergents such as zephan, cetavlon, phemerol, laurodin.

- **Anionic detergents** are detergents that release negative charged ions when ionized in water. It improves the wetting ability of the water by lowering the surface tension and melts the lipid in the cell wall. **The effects are usually on gram-positive bacteria.** It is weakly effective on Gram negatives. This group contains soaps, sodium lauryl sulfate and alkyl benzene sulphonate.

- **Nonionic detergents**; detergents in this group have very weak antiseptic and disinfectant effects. Bacteria in the skin are saponified (saponifying the lipid material by entering into microorganisms). Thus, washing hands with soap causes the microorganisms to run off. This group includes polyether and polyglycerol esters.

Disinfectants according to degree of influence to microorganisms

- High level disinfectants
- Moderate level disinfectants
- Low level disinfectants

- High level disinfection; Used for materials that are not resistant to sterilization methods and used in invasive procedures (surgical instruments with non-autoclaving plastic and other components). It **inactivates all microorganisms except a very resistant part bacterial spores.**
- Gluteraldehyde, hydrogen peroxide, peracetic acid and chlorine compounds

Chemical Sterilization- critical tools

Chemical sterilant	time	heat
Glutaraldehyde (% > 2.0)	10 h	20-25°C
Hydrogen peroxide-HP (7.5 %)	5 h	20-25°C
Peracetic acid-PA (0.2 %)	12 min.	50-56°C
HP (1.0 %) + PA (0.08 %)	8 h	20°C
HP (7.5 %) + PA (0.23 %)	3 h	20°C
HP (8.3 %) + PA (7 %)	5 h	25°C
Glut (1.12 %) + Phenol/phenate (1.93%)	12 h	25°C
Glut (3.4 %) + Isopropanol (26 %)	10 h	-20°C

FDA, September, 2006

High level disinfection- semi-critical tools

Disinfectant	Concentration
Glutaraldehyde	% >2.0
Ortho-phthalaldehyde (OPA)	0.55 %
Hydrogen peroxide*	7.5 %
Hydrogen peroxide+ Peracetic acid*	1.0 / %0.08 %
Hydrogen peroxide+ Peracetic acid*	7.5 %/ 0.23 %
Hypochlorite (free chlorine) *	650-675 ppm
Glutaraldehyde+ phenol / phenate **	1.21 % /1.93 %
*Can make cosmetic and functional damage** Activity not verified	

- Moderate (Intermediate) disinfection; is a level of disinfection that has **no effect on bacterial spores but is effective against mycobacteria, non-enveloped viruses and other microorganisms.** It is used on surfaces or devices where there is no possibility of contamination with bacterial spores and other highly resistant organisms and for semi-critical tools and equipment. Flexible fiberoptic endoscopes, laryngoscopes, vaginal specula ...
- Alcohols, iodophor compounds, phenol compounds

Moderate Disenfection- semi-critical tools

Disinfectant	Concentration
Ethyl or isopropyl alcohol	60-95 (70) %
Phenol and phenol compounds	0.4-5 %
Iodophors	30-50 ppm serbest iyot
Glucoprotamine	4 %

- **Low level disinfection**; is a level of disinfection that is ineffective against bacterial spores, mycobacteria and enveloped viruses, but can **affect some vegetative microorganisms.** It is used for non-critical instruments such as blood pressure measuring device, Electrocardiogram electrodes, stethoscope, etc.
- Quaternary ammonium compounds

%

Low level disinfection – non-critical tools and surfaces

Disinfectant	Concentration*
Ethyl or isopropyl alcohol	% < 50
Phenol and phenol compounds	0.4-5
Iodophors	30-50 ppm serbest iyot
Sodium hypochlorite	100 ppm serbest klor
Quaternary ammonium compounds	0.4 - 1.6 %
*Contact time>1 min	

Disinfectants according to the mechanism of action

- Disinfectants that disrupt the function of the cell membrane
- Disinfectants that denature cell proteins
- Disinfectants affecting nucleic acid
- Disinfectants affecting the function of microorganism enzymes
- Disinfectants affecting bacterial spores

1- Disinfectants that disrupt the function of the cell membrane

These disinfectants are affected by the **disruption of energy metabolism**, the semi-permeability of the membrane and the active transport

Surface active disinfectants: These substances are classified as cationic, anionic and nonionic depending on their ionization properties. The group of cationic disinfectants contains benzalkonium chloride, the anionic group contains soaps and fatty acids. **Benzalkonium chloride and soap are not used together because they neutralize each other.**

Phenol and Its Derivatives: It adheres to the cytoplasmic membrane and irreversibly inactivates oxidase and dehydrogenase enzymes. The other effect is to denature cell proteins. Phenol, methyl phenol, lysol, resorcinol, hexa-chlorophene, chlorhexidine.

3-5% phenol used in disinfection, 0.5% phenol used for vaccine and serum.

Organic Solvents: They disrupt the lipid structure of the cell membrane and also denature cell proteins. Alcohols, chloroform, ether, toluene.

2- Disinfectants that denature cell proteins

- These substances are effective by **disrupting the three-dimensional structure of the proteins and causing random ringing and healing of the polypeptide chain.**
- Alcohol, acetone, organic solvents

3- Disinfectants affecting nucleic acid

- Many **dyes** used in microbiology are in this group. The main ones are crystal violet, malachite green, brilliant green, fucsin, methylene blue and acridine. These dyes act as disinfectants by making compounds with nucleic acids and disrupting their activities. Methylene blue, acridine dyes are used as disinfectants on mucous membranes.

4- Disinfectants affecting the function of microorganism enzymes

- **Heavy metal salts:** Mercury, silver, copper salts. Their effects arise from the combination of the sulfhydryl groups of the enzymes. Mercury compounds today are rarely used because of their significant side-effects and low efficacy as antiseptics. Merthiolate and mercurochrome are used as skin disinfectants. The 1% solution of silver nitrate is used as an eye antiseptic for newborns.

- **Oxidizing substances:** Hydrogen peroxide, potassium permanganate, ozone affects the enzyme activity with oxidizing effect. Chlorine and chlorine donors from halogens (sodium hypochlorite, chloramines), bromine and iodine compounds are disinfectants with strong oxidizing effects. Chlorine and ozone are used in water disinfection.

- **Alkylating agents:** This group includes formalin, ethylene oxide and betapropiolactone. Formalin (37-40% solution of formaldehyde) has a killing effect on all microorganisms at high concentration. It is used to storage cadavers and tissues.

- **Ethylene oxide**; It is commonly used in sterilization. Liquid below 10.8 ^{o}C and gas above. It is used in mixture with 90% CO_2 due to its being flammable. Ethylene oxide gas affects both proteins and DNA. It affects all bacteria and spores, viruses a fungi. It has the ability to sterilize the contents inside the plastic packaging.

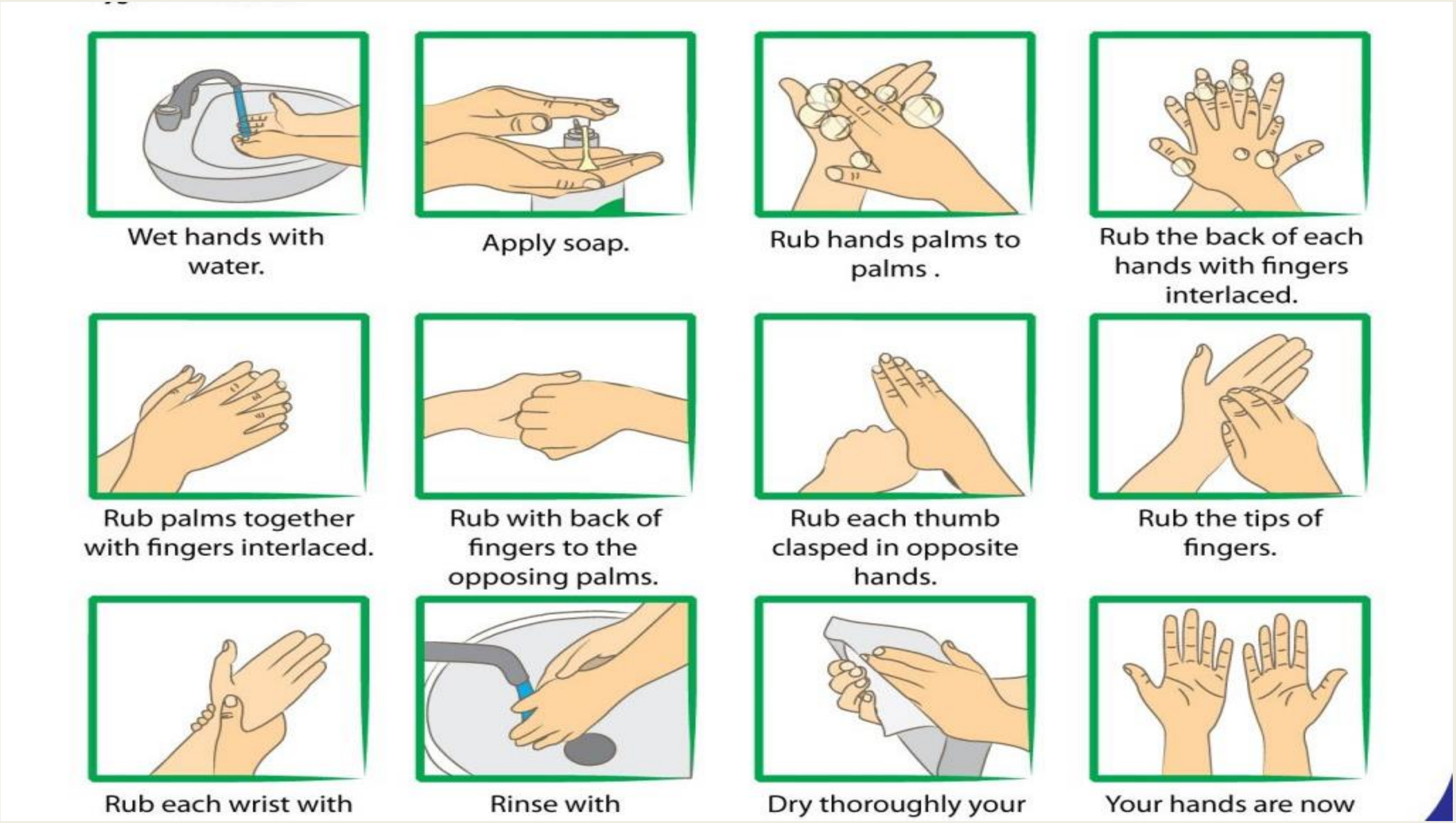
5- Disinfectants affecting bacterial spores

- The vegetative forms of spore bacteria are killed with disinfectant and they are prevented from doing sports again.
- The quaternary ammonium components are effective at the germination stage. Phenol acts in the phase of the formation of sporulation. Gluteraldehyde, formaldehyde, hypochlorite, iodine, hydrogen peroxide and ethylene oxide are effective in the mature spore phase.

CLINICAL PRACTICES OF DISINFECTION

Hand antisepsis

- Soaping for 15 seconds in daily life is enough for disinfection of hands.
- It is more effective to use 3% hexachlorophene or 5% cresol soap in handwashing of health personnel directly related to patients.
- Because they neutralize each other, soap should not be used together with benzalkonium chloride.
- Patient-related persons must soap their hands before and after approach to any procedure.
- Cleaning prior to surgical procedures requires different rules.


Wet hands with water.
Apply soap.
Rub hands palms to palms .
Rub the back of each hands with fingers interlaced.
Rub palms together with fingers interlaced.
Rub with back of fingers to the opposing palms.
Rub each thumb clasped in opposite hands.
Rub the tips of fingers.
Rub each wrist with
Rinse with
Dry thoroughly your
Your hands are now

Disinfection of floor, wall and goods: 3-5% phenol, 5% cresol.

Room disinfection: For this purpose, 10% formalin can be used. Formal gas can permeate everywhere, killing all microorganisms in the environment, including sports. Due to the difficulty of use, it is only applied in special cases. If all the bacteria in the environment are required to die, the room should be kept closed for 24 hours. Then the room is ventilated and the effect of formol gas is removed by application of ammonia. The room could be used later.

Skin and wound antisepsis

- Iodine tincture (2% iodine, 2.4% sodium iodide, 50% alcohol) is used for skin antisepsis. it is cleaned with alcohol to reduce irritation. Bodies are first extracted from dust and dust contaminated with the soil. It is then washed with soapy water or 1% bezalkalkonium chloride and 3% hydrogen peroxide. Also it is wiped Iodine tincture, 0.1% mertiolate or alcohol.

Laboratory disinfection

- 5% phenol, 5% cresol, 3% lysol. The pipettes and slides should be placed in a solution containing 2.4% hypochlorite.

Common used Disinfectant and Antiseptics

Disinfectant and Antiseptics	**Usage area**
Phenol	Disinfection of laboratory equipments, pipettes, swabs, operation rooms
Formalin	Disinfection of materials and rooms, preservation of tissues and cadavers
Alcohol	Skin and wound antisepsis, disinfection of some materials
Hydrogen peroxide	Skin and wound antisepsis, disinfection of some materials
Glutaraldehyde	Disinfection of materials and rooms, disinfection of surgical materials
Heksaklorofen	Skin antisepsis
Sodium hypocholorite	Disinfection of various items, laundries and the environment, Water disinfection.
Iodine compounds	Skin antisepsis, Disinfection of some materials
Sulfuric acid	Water pipe disinfection

Common used Disinfectant and Antiseptics-2

Disinfectant and Antiseptics	Usage area
Boric acid	Eyes antisepsis
Cresol	Disinfection of surfaces
Lysol	Skin antisepsis, Disinfection of hospital environment
Lugol	Skin and mucosa antisepsis
Chlorine	Water disinfection
Potassium permanganate	Skin antisepsis
Quicklime (Calcium oxide)	Cadavers
Ethylene oxide	Chemical sterilization and disinfection
Soaps and detergents	Mechanical cleaning
Merthiolate	Skin and wound antisepsis
Silver nitrate	Eyes antisepsis

Activity Tests

- Various methods have been developed to determine the effectiveness of disinfectants.
- TSE is used in Turkey

Tests used for measurement of effectiveness;

According to test organisms; They are classified as

- Antibacterial
- Antifungal
- Antiviral

In-vitro tests

- **Suspension tests**

  * Qualitative suspension tests (with or without colony in the passage)

  * Quantitative suspension tests (a test based on the comparison of the number of viable microorganisms in the first inoculum with the number of microorganisms after contact with the disinfectant.

- Phenol Coefficient Test (Rideal-Walker): A qualitative method based on the measurement of disinfectant activity compared to phenol.

- **Capacity tests:** The most advanced test in this group is the **Kelsey Sykes** test. By adding bacteria several times to the disinfectant, the bactericidal killing capacity of the disinfectant is observed. For this purpose, a contaminated material or device is disposed in to the disinfectant. Preservation of activity against increased microorganisms is indicative of disinfectant capacity.

- **Carrier tests:** An important test in evaluating preparations designed for instrument disinfection. Metal, catheter parts are artificially contaminated and immersed in the used disinfectant dilution. After a certain period of contact, it is tested whether the bacteria die or not.

- **Practice tests:** Applied tests are second-phase tests performed in real-life condition. In some countries this test is applied for each application area, including tools and surfaces, room corners, air, sputum, faeces, hand and skin, swimming pool and others.

- The general principle in all efficacy tests is that the dilutions of the disinfectant substance to be tested are compared with certain microorganisms. At the end of the contact period, it is determined how much microorganisms are alive, how many have died. All tests performed are "Dilution-Neutralization Method".

Principles of Dilution-Neutralization Method

- The offending agent is mixed with the bacterial suspension.
- Disinfectant is added.
- At the end of the contact times it is mixed with the neutralizing agent.
- After the neutralization period, the specimens are inoculated into the solid medium.
- At the end of the incubation period, bacteria are counted.
- As a control, the activity of bacterial suspension, disrupter, diluent liquid should be tested alone.

- The acceptability of the disinfectant effect is related to the Reduction Factor (RF) value.
- Reduction Factor (RF) is the difference between the log of the number of microorganisms prior to disinfectant exposure and the logarithm of the number of viable microorganisms after treatment with disinfectant.
- The number of microorganisms in the beginning should be 10^9 / ml or more.
- Usually a reduction of 5 log RF is required after 1 minute of contact.

Antibacterial activity test

- *Pseudomonas aeruginosa* ATCC 15442
- *Escherichia coli* ATCC 10536
- *Staphylococcus aureus* ATCC 6538
- *Enterococcus hirae* ATCC 10541
- *Salmonella typhimurium* ATCC 13311 strains are recommended.

Fungucidal activity test

- *Candida albicans* ***ATCC 10231,***
- *Aspergillus niger* ***ATCC 16404*** Malt Extract Agar(MEA)
- The vegetative cells of *C.albicans*, The spore of *A.niger*
- 10^4 or more decrease in viability at 60 minutes

- Experiments are performed separately for each microorganism.
- It is tried for 1, 5, 15, 30, 45 or 60 minutes.
- Controls should also be done.
- The number of microorganisms at the beginning of the experiment is absolutely determined and the ratio of microorganisms decreasing after the main test is evaluated.