Lab.N0-1

General Principals of Clinical Laboratory Analysis

First: Elimination of Microbes.
Second: Laboratory Equipments and Instruments.
Third: Laboratory specimens.
Forth: Safety precautions.
Fifth: Laboratory Test.

First: Elimination of Microbes:

Control of Microorganisms achieved by means of Mechanical, Physical and chemical agents. Physical agents include methods of control like (high or low temperature, desiccation, osmotic pressure, radiation, and filtration).The chemical agents refers to the use of (Disinfectants, Antiseptics, Antibiotics, and Chemotherapeutic antimicrobial agents). (See table below).

CONTROL OF MICROORGANISMS is essential in order to prevent the transmission of diseases and infection, stop decomposition and spoilage, and prevent unwanted microbial contamination to laboratory staff and equipments and it is done by the following:

Disinfection and decontamination

- CONTAMINATION – introduction of microorganisms into tissues or sterile materials.
- DECONTAMINATION – the removal or inactivation of biological agents by physical or chemical means
- DISINFECTANT – an agent, usually chemical, that inactivates viruses or kills vegetative bacteria but not necessarily resistant forms such as spores
- ANTISEPTIC – a substance that prevents or arrests the growth or action of microbes, either by inhibiting their activity or by destroying them
- SANITIZER – an agent that reduces the numbers of vegetative bacteria only
- STERILIZER – process or agent, physical or chemical that destroys or eliminates all forms of life, including spores.

1. **Sterilization**: is the process of destroying all living organisms and viruses. A sterile object is one free of all life forms, including bacterial endospores, as well as viruses.
2. **Disinfection**: is the elimination of microorga nisms from inanimate objects or surfaces.
3. **Decontamination**: is the treatment of an object or inanimate surface to make it safe to handle.
4. **Disinfectant**: is an agents used to disinfect inanimate objects but generally to toxic to use on human tissues.
5. **Antiseptic**: is an agent that kills or inhibits growth of microbes but is safe to use on human tissue.
6. **Sanitizer**: is an agent that reduces, but may not eliminate, microbial numbers and Instruments

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

1. They may damage the lipids and/or proteins of the semi permeable cytoplasm membrane of microorganisms resulting in leakage of cellular materials needed to sustain life.
2. They may denature microbial enzymes and other proteins, usually by disrupting the hydrogen and disulfide bonds that give the protein its structure.

DISINFECTION AND DECONTAMINATION

A- Mechanical	**B- Physical**	**C- Chemical**	**C- Chemical cont.**
Cleaning (soap and water)	Radiation	Liquid	Gases & Vapor-Phase
Filtration	Heat	▪ Alcohols	▪ Chlorine dioxide
	▪ Autoclave(steam + pressure	▪ Acids/alkalis	▪ Formaldehyde
	▪ Steam	▪ Aldehydes	▪ Hydrogen peroxide
	▪ Dry Heat (‘quats’)	▪ Peroxides/oxidizers)	
	▪ Boiling	▪ Phenols	
		▪ Chlorines/Iodines	
		▪ Quaternary ammonia	

Elimination of Microbes:

A- Mechanical methods:

- Cleaning (soap and water).
- Filtration.
  Microbiological membrane filters provide a useful way of sterilizing materials such as vaccines, antibiotic solutions, animal sera, enzyme solutions, vitamin solutions, and other solutions that may be damaged or denatured by high temperatures or chemical agents. The filters contain pores small enough to prevent the passage of microbes but large enough to allow the organism-free fluid to pass through. The liquid is then collected in a sterile flask.

B- Physical Methods:

- **Radiation**

1. Ultraviolet Radiation

The microbicidal activity of ultraviolet (UV) light depends on the length of exposure: the longer the exposure the greater the cidal activity. It also depends on the wavelength of UV used. The most cidal wavelengths of UV light lie in the 260 nm - 270 nm range where it is absorbed by nucleic acid.

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

2. Ionizing Radiation

Ionizing radiation, such as X-rays and gamma rays, has much more energy and penetrating power than ultraviolet radiation. It is often used to sterilize pharmaceuticals and disposable medical supplies such as syringes, surgical gloves, catheters, sutures, and petri plates.

- **.Heat or Temperature**:
  Microorganisms have a minimum (optimum), and maximum temperature for growth. Temperatures below the minimum usually have a static action on microorganisms. They inhibit microbial growth by slowing down metabolism but do not necessarily kill the organism, and temperatures above the maximum usually have a cidal action, since they denature

microbial enzymes and other proteins. Temperature is a very common and effective way of controlling microorganisms which include:

a- Moist heat

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

1. Pasteurization:

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

2. Autoclave

Autoclaving employs steam under pressure. Water normally boils at 100°C; however, when put under pressure, water boils at a higher temperature r 15 pounds per square inch of pressure 121°C, kill bacterial endospores.

3. Boiling water

Boiling water (100°C) will generally kill vegetative cells after about 10 minutes of exposure.

b- Dry heat

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

1. Hot air sterilization (oven):

Microbiological ovens employ very high dry temperatures: 171°C for 1 hour; 160°C for 2 hours or longer; or 121°C for 16 hours or longer depending on the volume. They are generally used only for sterilizing glassware, metal instruments.

2. Incineration:

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

c- Low Temperature:

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

d- Desiccation

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

e- Osmotic Pressure:

Hypotonic and Isotonic environments are not usually harmful to microorganism but hypertonic environments are more effective for killing microorganism by increasing salt and sugar concentration.

C- Chemical Method:

1. Phenol and phenol derivatives
2. Soaps and detergents
3. Alcohols
4. Acids and an alkaline 5. Heavy metals
6. Chlorine
7. Iodine
8. Aldehydes.
9. Ethylene oxide gas

Factors which may influence antimicrobial activity when we are choosing a method of controlling microorganisms.

1. The concentration and kind of a chemical agent used;
2. The intensity and nature of a physical agent used; 3. The length of exposure to the agent;
4. The temperature at which the agent is used;
5. The number of microorganisms present
6. The organism itself, and
7. The nature of the material bearing the microorganism.