Lec (3-4) **Genetic and Sterilization** **Dr. Marwa Kadhim**

Introduction

Genetics: Genetics is the study of genes, their structure and function, heredity and variation.

Genomics: The study and analysis of the nucleotide sequence of DNA is called genomics.

Genome: The complete set of genetic information for a cell is referred to as its genome.

Structure and Functions of the Genetic Material

Nucleic Acid Structure

A substance called **deoxyribonucleic acid (DNA)** is the substance of which genes are made , another substance called *ribonucleic acid (RNA)*, are together referred to as nucleic acids.

Nucleotides:

Nucleotides are the structural units of nucleic acids. Nucleotides are named according to their nitrogenous base.

Parts of Nucleotide

Each **nucleotide** has three parts:

i. A nitrogen-containing base

Purines and pyrimidines: The nitrogen containing bases are cyclic compounds made up of carbon, hydrogen, oxygen, and nitrogen atoms. The bases are named adenine (A), thymine (T), cytosine (C), guanine (G), and uracil (U). A and G are double-ring structures called **purines,** whereas T, C, and U are single ring structures referred to as **pyrimidines.**

ii. **A pentose (five-carbon) sugar called deoxyribose or ribose**

iii. **A phosphate group (phosphoric acid):**

A. Deoxyribonucleic Acid (DNA)

Structure

Double helix: According to the model proposed by Watson and Crick, a DNA molecule consists of two long strands wrapped around each other to form a double helix (Fig-1). The double helix looks-like a twisted ladder, and each strand is composed of many nucleotides. The two strands are held together by weak hydrogen bonds between the nitrogenous bases of the opposing strands.

Sugar-phosphate backbone: Every strand of DNA composing the double helix has a "backbone" consisting of alternating deoxyribose sugar and phosphate groups. The deoxyribose of one nucleotide is joined to the *phosphate group* of the

next. The nitrogen containing bases make up the rungs of the ladder. Purine A is always paired with the pyrimidine T and that the purine G is always paired with the pyrimidine C. The bases are held together by hydrogen bonds; A and T are held by two hydrogen bonds, and G and C are held by three hydrogen bonds.

Base pairing: The characteristic bonding of A to T and G to C is called base pairing .

Complementary: Because the sequence of bases of one strand is determined by the sequence of bases of the other, the bases are said to be **complementary.**

Code and Codon

Genetic information is stored in DNA as a **code**. The unit of code is known as **codon.** It consists of a sequence of three bases. Therefore, code is triplet.

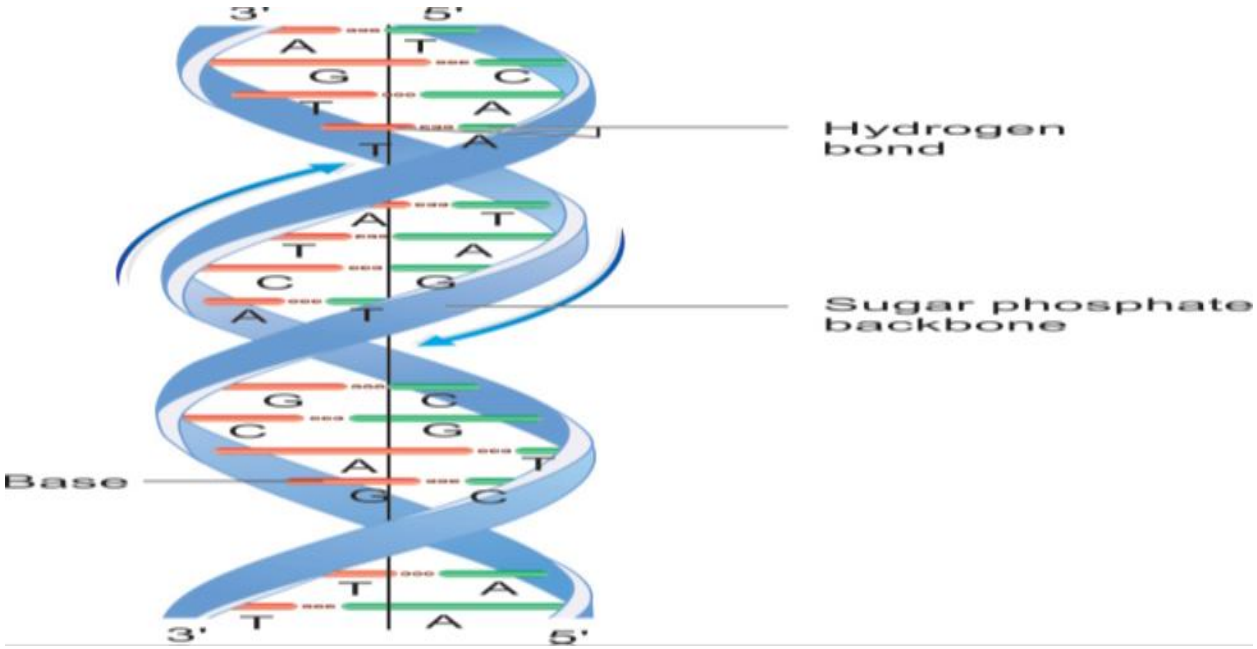
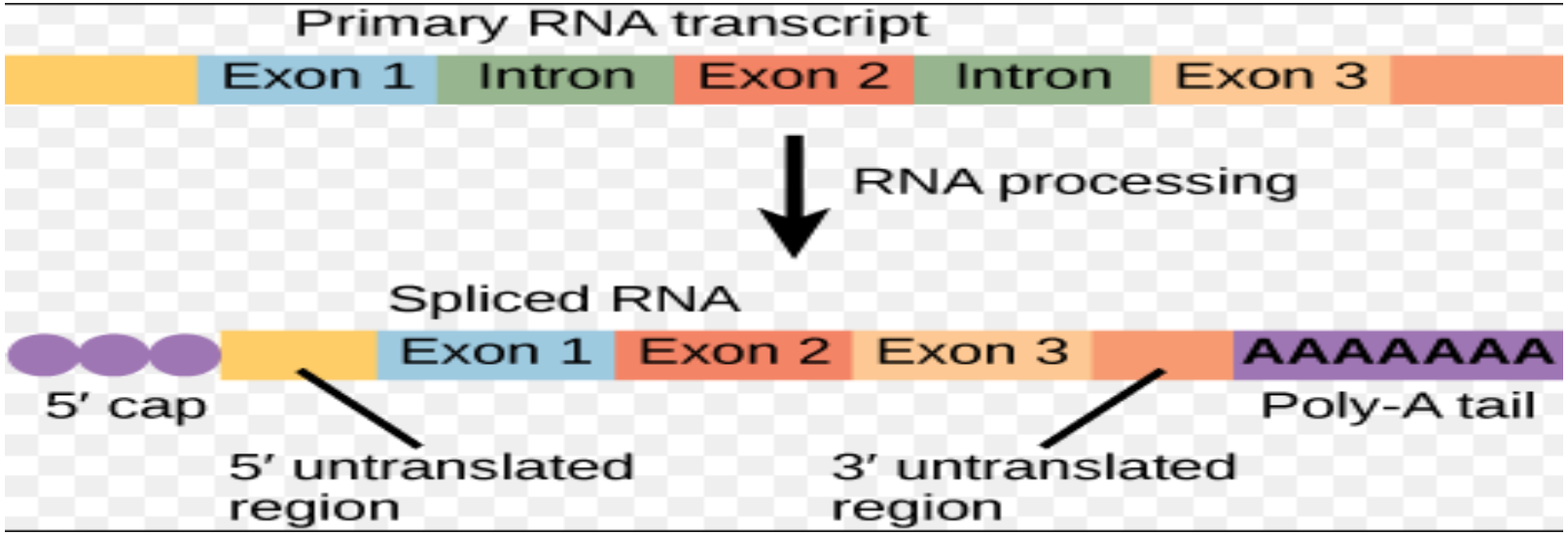


Figure -1: A schematic drawing of the Watson-Crick structure of DNA, showing helical sugar phosphate backbones of the two strands held together by hydrogen bonding between the bases

Gene

A segment of DNA carrying a number of codons specifying for a particular polypeptide is known as **gene.** A large number of genes constitute a *locus* . A DNA molecule consists of a large number of genes, each of which contains hundreds of thousands of nucleotides. The bacterial chromosome consists of a double-stranded molecule of DNA arranged in a circular form.

Introns and exons: In higher forms of life, several stretches of DNA that do not appear to function as codons occur between the coding sequences of genes. These apparently useless noncoding intrusions are called **introns,** while the stretches of coded genes are called **exons.**



INTRONS
VERSUS
EXONS

INTRONS	EXONS
Introns are the DNA segments which do not encode any amino acid sequence in the coding region	Exons are the DNA segments which encode a part of an amino acid sequence of a complete protein
Belong to the non-coding DNA	Belong to the coding DNA
Considered as the bases located between two exons	Considered as the bases which encode an amino acid sequence of a protein

B. Ribonucleic Acid (RNA) Structure

Three major kinds of RNA have been identified in cells. These are referred to as messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). Each type of RNA has a specific role in protein synthesis.

Gene Expression

Gene expression involves two separate but interrelated processes transcription and translation.

A. Transcription: Transcription is the process of synthesizing RNA from a DNA template. The DNA acts as a template for the transcription of RNA by RNA polymerase for subsequent protein production within the cell. RNA polymerase attaches itself to the beginning of a gene on DNA and synthesizes mRNA, using one of the strands in DNA as a template. This process is known as transcription. The bases in mRNA will be complementary to one strand of DNA since DNA acts as a template for synthesis of mRNA.

B. Translation: Translation is the process of decoding the information carried on the mRNA to synthesize the specified protein.

Process of translation: The process of translation requires three major components—mRNA, ribosomes, and tRNAs, in addition to various accessory proteins.

Messenger RNA (mRNA): The mRNA is a temporary copy of genetic information. It carries the coded information for making specific proteins from DNA to ribosomes, where proteins are synthesized.

Ribosomes: Serve as the sites of translation, and their structure facilitates the joining of one amino acid to another.

Transfer RNA (tRNA): The tRNA molecule contains a triplet at one end and amino acid at the other end. The ribosome moves along the mRNA until the entire mRNA molecule has been translated into corresponding sequences of amino acids. Finally, the sequence of amino acids in the resulting polypeptide chain determines the configuration into which the polypeptide chain folds itself, which in many cases determines the enzymatic properties of the completed protein.

Extra chromosomal genetic Elements

Plasmids

Most bacteria possess extra chromosomal genetic element in addition to chromosomal DNA elements known as **plasmid**. It consists of a circular piece of double-stranded DNA, can replicate autonomously (independent replicons) and can maintain in the cytoplasm of a bacterium for many generations.

Mutation

It is a random, undirected, heritable variation caused by an alteration in the nucleotide sequence at some point of the DNA of the cell.

Types of Mutation

Mutations can be divided conveniently into:

A. Spontaneous mutation: Many mutations occur spontaneously in nature in the absence of any mutation-causing agents.

B. Induced mutation: The frequency of mutation is greatly enhanced by exposure of cells to several agents (mutagens) which may be physical or chemical.

Mutagens

1. **Physical agents:** (i) UV rays; (ii) lionizing radiation, e.g. X-rays; (iii) Visible light; (iv) Heat

2. Chemical agents*:* (i) Alkylating agents; (ii) Acridine dyes; (iii) 5-Bromouracil

C. Point mutations: Point mutations affect just one point (base pair) in a gene. Such mutations may be a change to or substitution of a different base pair. Alternatively, a point mutation can result in the deletion or addition of a base pair. It is, in general, reversible and is of two classes:

1. Base pair substitution: This comprises those mutants in which a single base pair (nucleotide) has been substituted for another pair, and can be subdivided into transition (one purine is replaced by other purine or a pyrimidine is replaced by other pyrimidine) and transversion (substitution of a purine for a pyramidine and

vice versa in base pairing

2. Base pair deletion or insertion

Frame shift mutations: If the number of bases inserted or deleted is not a multiple of three, there will be shift in the reading frame, i.e. frame shift mutations.

Importance of Bacterial Mutation

Drug resistance and development of live vaccines: The practical importance of bacterial mutation is mainly in the field of drug resistance and development of live vaccines.

Transmission of genetic material (gene transfer)

DNA may be transferred between bacteria by the following mechanisms:

A. Transformation

B. Transduction

C. Lysogenic conversion

D. Conjugation.

A. Transformation

Transformation is the transfer of genetic information through the agency of free *("naked")* DNA The initial experiment on transformation was performed by Frederick Griffith in England in 1928. Griffith's Experiment Demonstrating Genetic Transformation

i. Griffith (1928) found that injections of living encapsulated bacteria killed the mouse (Fig.3).

ii. Injections of live non encapsulated bacteria or dead encapsulated bacteria did not kill the mouse.

iii. When the dead encapsulated bacteria were mixed with live non encapsulated bacteria and injected into the mice, many of the mice died. In the blood of the dead mice, Griffith found living, encapsulated bacteria. Hereditary material (genes) from the dead bacteria had entered the live cells and changed them genetically so that their progeny were encapsulated and therefore virulent.

Transformation and Bacteria: Transformation occurs naturally among very few genera of bacteria, including *Bacillus*, *Haemophilus*, *Neisseria*, *Acinetobacter*, and certain strains of the genera *Streptococcus* and *Staphylococcus*.

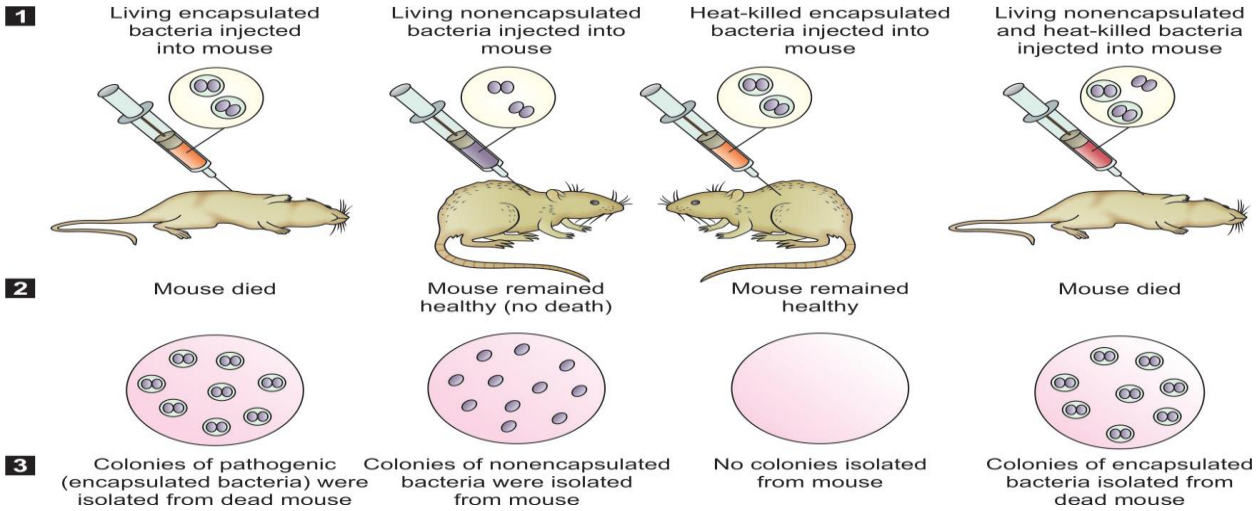


Figure -3 :Transformation experiment of Griffith

B. Transduction

The transfer of a portion of the DNA from one bacterium to another by a bacteriophage is known as transduction. Bacteriophages are viruses that parasitize bacteria and consist of a nucleic acid core and a protein coat. Most bacteriophages carry their genetic information (the phage genome) as a length of double-stranded DNA coiled up inside a protein coat. When bacteriophages multiply inside an infected bacterial cell, each phage head is normally filled with a copy of the replicated phage

genome. During the assembly of bacteriophage progeny inside infected bacteria, 'packaging errors' may occur occasionally. A phage particle

may have at its core a segment of the host DNA besides its own nucleic acid. When this particle infects another bacterium, DNA transfer is affected and the recipient cell acquires new characteristic coded by the donor DNA. Bacterial genes have been transduced by the phage into the second cell .(fig-4)

Types of Transduction

Two major types of transduction are known to occur in bacteria: Generalized transduction and specialized transduction.

1. Generalized transduction: Since phages of this type pick up any portion of the bacterial chromosome at random are termed generalized transducing phages.

2. Specialized or restricted transduction: A specific bacteriophage

transduces only a particular genetic trait.

Role of Transduction

1. In plasmids.
2. Penicillin resistance in staphylococci: The plasmids determining penicillin resistance in staphylococci are transferred from cell to cell by transduction.
3. Genetic mapping of bacteria.
4. Treatment of some inborn metabolic defects.

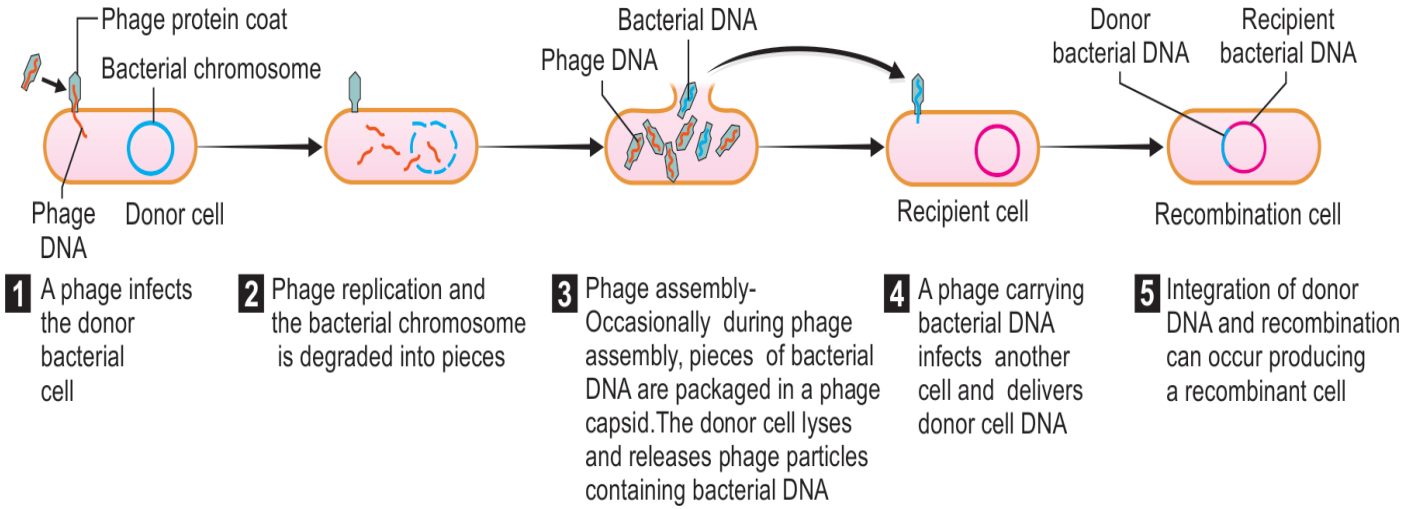


Figure -4: Transduction by a bacteriophage (showing generalized transduction)

C. Lysogenic Conversion

Bacteriophages exhibit two types of life cycle

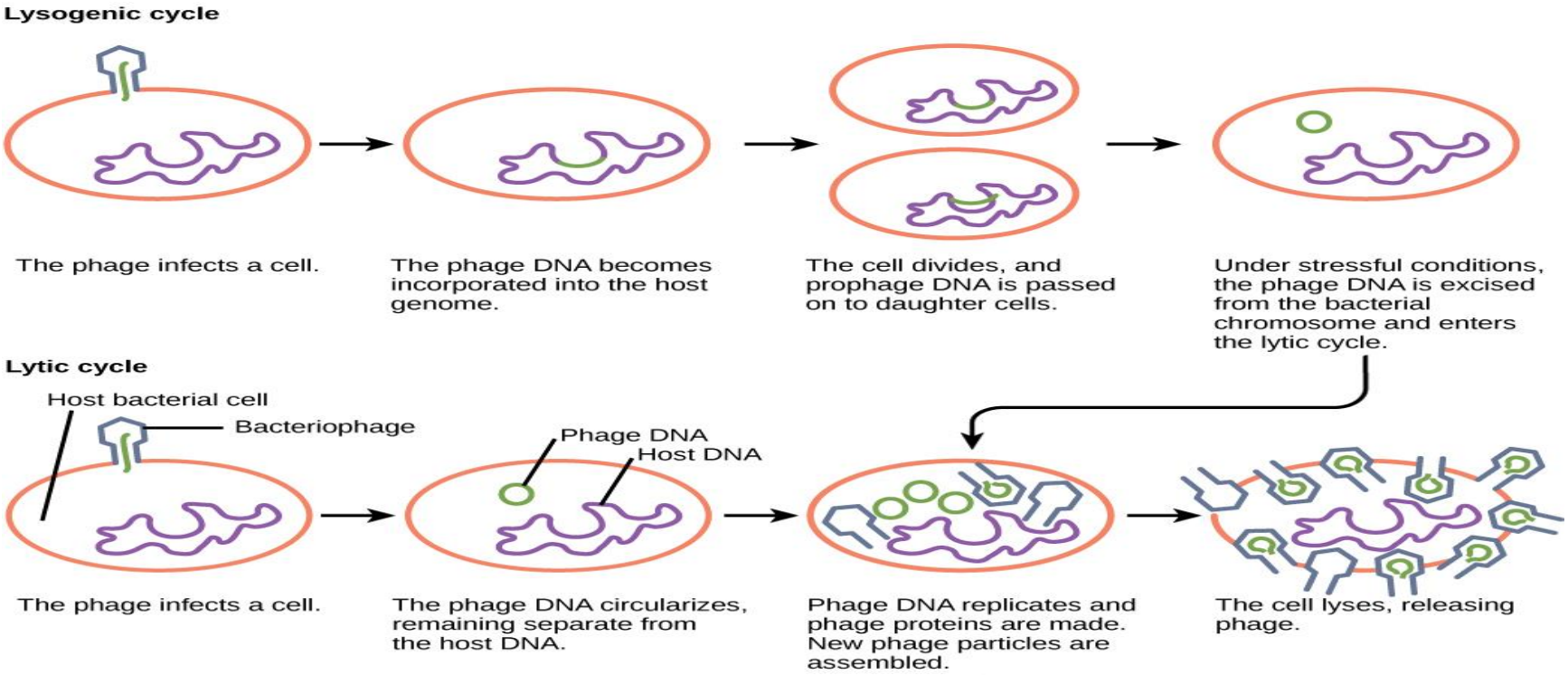
i. Virulent or lytic cycle: In the virulent or lytic cycle, large numbers of progeny phages are built up inside the host bacterium, which ruptures to release them.

ii. Temperate or non lytic cycle: In the temperate or non lytic cycle, the host bacterium is unharmed. The phage DNA becomes integrated with the bacterial chromosome as the prophage and is replicated stably as part of the host cell chromosome and is transferred to the daughter cells. This process is called **lysogeny** and bacteria harboring prophages are called **lysogenic** bacteria.

Medical Importance

1. Toxigenicity in *diphtheria bacilli:* Of great medical importance is the lysogenic conversion in *diphtheria bacilli*, which acquire toxigenicity (and therefore virulence) by lysogenization with the phage beta.

2. Production of *staphylococci, streptococci* and *Clostridia* toxins is also dependent upon lysogenic conversion by specific bacteriophages.



D. Conjugation

Conjugation is a process in which one cell, the donor or male cell, makes contact with another, the recipient or female cell, and DNA is transferred directly from the donor into the recipient. Lederberg and Tatum (1946) first described bacterial conjugation in a strain of *E. coli* called K12.

Types of Conjugation

Three types of conjugation are described below:

1. Plasmid Transfer

2. Chromosomal Transfer

3. Plasmid and Chromosomal Transfer

Applications of Genetic Engineering

1. Production of vaccines*:* Foot and mouth disease, hepatitis B, rabies viruses and DNA vaccine.
2. Production of proteins of therapeutic interest*:* Human growth hormones, human insulin, erythropoietin, blood-clotting factor VIII, tissue plasminogen activator, interferons, tumor necrosis factor, interleukin-1, 2, and 3, granulocyte colony stimulating factor, epidermal growth factor, fibroblast growth factor.
3. Gene therapy.
4. Others: It has also become essential to laboratory diagnosis, agriculture, and many other disciplines.

Sterilization

Definitions of frequently used terms

Sterilization: refers to any process that eliminates, removes, kills, or deactivates all forms of life and other biological agents (such as fungi, bacteria, viruses, spore forms .

Disinfection: are antimicrobial agents that are applied to the surface of non-living objects to destroy microorganisms that are living on the objects. Disinfection does not necessarily kill all microorganisms, it is less effective than sterilization.

Antiseptics: Antiseptics are chemical agents applied to the tissue to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population.

Applications of Sterilization and Disinfection

1. **Aseptic techniques:** Used in microbiological research, the preservation of food and the prevention of the disease.
2. **Sterile apparatus and culture media:** Laboratory work with pure cultures requires the use of sterile apparatus and culture media.

Methods of Sterilization and Disinfection

A. Physical agents

B. Chemical agents

A. Physical Agents

1. Sunlight

Sunlight has an appreciable bactericidal activity. Its disinfectant action is primarily due to its content of ultraviolet rays.

2. Drying

Drying in air has a deleterious effect on many bacteria.

3. Heat

Either dry or moist heat may be applied. Materials that may be damaged by heat can be sterilized at lower temperature, for longer periods or by repeated cycles.

a. Dry Heat Sterilization

i. **Red heat:** Inoculating wires, loops and points of forceps are sterilized by holding them almost vertically in a Bunsen flame until red hot.

ii. **Flaming:** Scalpel blades, glass slides, mouth of culture tubes and bottles are exposed to a flame for a few seconds without heating them to become red hot.

iii. **Incineration:** This is an efficient method for the sterilization and disposal of contaminated materials at a high temperature. Such as pathological waste materials, surgical dressings, contaminated material, animal carcasses and other clinical waste.

iv. **Hot air oven:** Hot air oven is the most widely used method of sterilization by dry heat. It is used to process materials which can withstand high temperatures for length of time needed for sterilization by dry heat.

Uses

1. **Glassware:** These should be perfectly **dry before being placed in the oven**.
2. **Test tubes and flasks**.
3. **Rubber materials**, except silicon rubber, will not withstand the sterilizing temperature.
4. **Heat-sensitive materials:** Dry heat sterilization is slow and not suitable for heat-sensitive materials like many plastic and rubber items.

b. Moist Heat Sterilization

Moist heat is divided into three forms:

A. At temperature below 100°C

B. At a temperature of 100°C

C. At temperature above 100°C

A. At temperature below 100°C: It includes:

1. Pasteurization of milk: Disinfection by moist heat at temperature below 100°C is termed **pasteurization.** Milk can be pasteurized in two ways. The temperature is employed either **63°C for 30 minutes (holder method)** or **72°C for 15–20 seconds (flash method)** followed by **rapid cooling to 13°C or lower.** All non sporing pathogens such as *Mycobacteria*, Brucellae and salmonellae are destroyed by these processes. ***Coxiella burnetii*** is relatively heat resistant and may survive the holder method.

2. **Vaccine preparatior:** Vaccines prepared from non sporing bacteria may be inactivated in a water bath at 60°C for one hour.

3. **Inspissation**: Media such as Lowenstein- Jensen and Loeffler's serum are rendered sterile by heating at 80-85°C for half-an hour on three successive days **(fractional sterilization)**. This process is called **inspissation** and instrument used is called **inspissator.**

4. **Water bath:** Washing or rinsing laundry or utensils in water bath at 70-80°C for few minutes will kill most non sporing microorganisms present.

B. At temperature of 100°C

1. **Boiling: Boiling** at 100°C for 10–30 minutes kills all vegetative spores and some bacterial spores.

2. **Steam at atmospheric pressure at 100°C for 90 minutes**

This can be provided by the traditional **Koch and Arnold steamer** (or by the multipurpose autoclave). **Koch and Arnold steamer (Fig. 1)**. They are exposed to steam at atmospheric pressure for 90 minutes. One single exposure to steam for 90 minutes ensures complete sterilization.

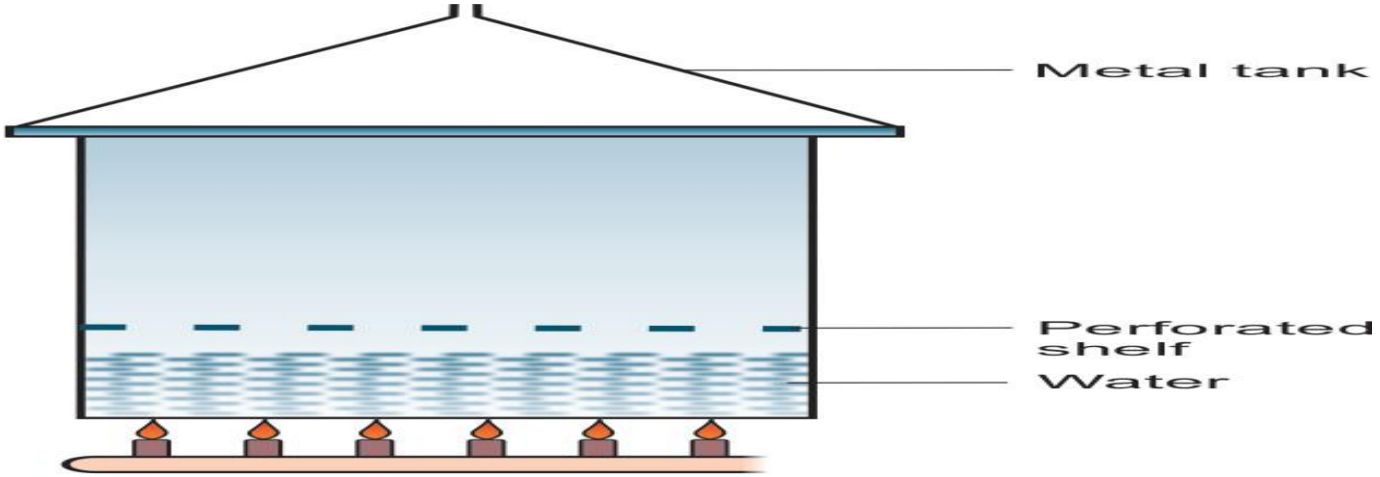


Fig-1: Steamer

3. **Tyndallization:** An exposure of steam at 100°C for 20 minutes on three successive days is called **tyndallization or intermittent sterilization.** This is a fractional method of sterilization. The instrument commonly used is Koch and Arnold steamer.

Uses: This method is useful in sterilizing heat-sensitive culture media containing such materials as carbohydrates, egg or serum, which are damaged by higher temperature of autoclave.

C. At temperature above 100°C Steam under pressure: Steam above 100°C or saturated steam is a more efficient sterilizing agent than hot air.

Autoclave

Autoclaving is the process of sterilization by saturated steam under high pressure above 100°C. Steam sterilization is carried out in a pressure chamber called an **autoclave** (a device somewhat like a fancy pressure cooker).

Uses

i. For sterilizing culture media and other laboratory supplies, aqueous solutions, rubber material.

ii. For all materials that are water-containing.

iii. Particularly useful for materials which cannot withstand the higher temperature of hot air oven.

4. Filtration

Filtration is the principal method used in the laboratory for the sterilization of heat labile materials, e.g. sera, solutions of sugars or antibiotics used for the preparation of culture media.

Uses

1. **Heat-sensitive solutions:** For sterilization of pharmaceuticals, ophthalmic solutions, culture media, oils, antibiotics and other heat-sensitive solutions.
2. **For separation of bacteriophages and bacterial toxins from bacteria.**
3. **Isolation of organisms which are scanty in fluids.**
4. **Concentration of bacteria from liquids,** e.g. in testing water samples for *cholera vibrios or typhoid bacilli*.
5. **For virus isolation.**

5. Radiation

Two types of radiations are used:

I. **Non-ionizing radiations:** Infrared and

ultraviolet rays are of non-ionizing type. The effectiveness of UV light as a lethal and mutagenic agent is closely correlated with its wavelength.

II. **Ionizing radiations:** These include X-rays, g (gamma) rays and cosmic rays. These have very high penetrative power and are highly lethal to all cells including bacteria. Ionizing radiations damage the DNA by various mechanisms.

Applications

i. **For sterilization in pharmacy and medicine**.

ii. **Sterilization of packaged disposable articles** such as plastic syringes, catheters and gloves that are unable to withstand heat. Since there is no appreciable increase in temperature in this method, it is known as **cold sterilization.**

B. Chemical Agents :

A. Agents that damage the cell membrane :- Surface active disinfectants ,Phenolic compounds and Alcohols

B. Agents that denature proteins 1. Acids and alkalies 2. Alcohols

C. Agents that modify functional groups of proteins and nucleic acids:

1. Heavy metals and their compounds 2. Oxidizing agents—Halogens– Hydrogen peroxide