

General Microbiology

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Introduction

- ✓ **Microbiology** is the study of living organisms of microscopic size, which include **bacteria, fungi**, the infectious agents at the border line of life that are called viruses and prions, infectious protein particles.
- It is derived from three Greek words (Micros = Small, Bios = life, Logos = study)
- It studies about their **structure, reproduction, physiology, metabolism and classification**

Subdivisions of microbiology

➤ It can be sub divided into: Virology (studies about viruses), Bacteriology (studies about bacteria), Mycology (studies about Fungi).

Viruses: The smallest infectious particles Ranging in diameter from 18 to 300 nm. They are consist of either DNA or RNA not both. They have living and non living characteristics.

- **Bacteria:** are prokaryotes, relatively simple in structure, reproduce by asexual division, covered by cell wall simple unicellular organisms without organelles like: Nuclear membrane, Mitochondria, Golgi bodies and Endoplasmic reticulum
- **Fungi:** are eukaryotes, that is why cellular structure is more complex and contain a well defined: Nucleus, Mitochondria, Golgi bodies, Endoplasmic reticulum,

- Fungi exist either in a unicellular form (Yeast) or in filamentous form (Mold)
- Veterinary microorganism deals with microbial agents affecting animals.

1.1.Historical development of microbiology

- **Theory of spontaneous generation :**
- **Theory of biogenesis**
- **Cell Theory**
- **Germ theory**
- **Golden Era of microbiology**

❖ Spontaneous generation

- They believed that life arises from non living matter.
 - Aristotle (384-332. B.C) thought some of the simple invertebrates could arise by spontaneous generation. Eg
 - Toads
 - Snake
 - Mice
- } could born from moist soil (mud)
- Maggots (the larvae of fly) could arise from decay

Theory of biogenesis:

- the theory states that all living things come from pre-existing cells, but not spontaneously
- The view of spontaneous generation was first challenged by Italian physician Francesco Redi (1626-1697) by doing a series of experiments on decaying meat and its ability to produce maggot spontaneously

Cond'

- Although Redi had given evidence for disproving the theory of spontaneous generation his experiment was challenged by J. NEDHAM's (1745) simple experiment

J.NEDHAM: -

- Cooked a piece of meat to destroy pre-existing cells
- Placed them in open flask and closed the flask firmly
- left them for few days
- Saw colonies of micro-organisms on the surface
- Concluded as life arise spontaneously
- Microbes grew because the flask was not properly sterilized.

- In 1609 **Galileo** had created a series of lenses in a tube to produce higher magnification.
- **Robert Hooke (1635 - 1703)** made many scientific discoveries in the 17th century. including making one of the first microscopes to see the details of the structure of plant cells (little boxes or “cells) concluded that life’s smallest structural units of cell (**cell theory**) states that all living things were composed of cells

- **Antony van Leeuwenhoek (1632-1723)** :made the first useful microscopes and he used such a microscope to see the first microscopic cells.

❖ **GERM THEORY OF DISEASE**

- A theory that states as Micro organisms might cause disease
- The first proof that bacteria actually cause disease came from Robert Koch in 1876

❖ **Golden Era of microbiology:**

- The years followed 1879 considered as golden ages of microbiology. This is because:
- Most bacterial pathogens were isolated.
- Progress was made in determining how animals resisted diseases
- Different techniques were developed for protecting humans and livestock against pathogens.

Prokaryotic and Eukaryotic cells

- Living cells, the smallest functional units of life capable of independent existence can be divided into two sharply differentiated groups: Prokaryotes and Eukaryotes

Prokaryotes

- Are primitive organisms that lack a clearly defined membrane-bound nucleus
- Their genetic information is contained in a single circular chromosome.

Eukaryotes

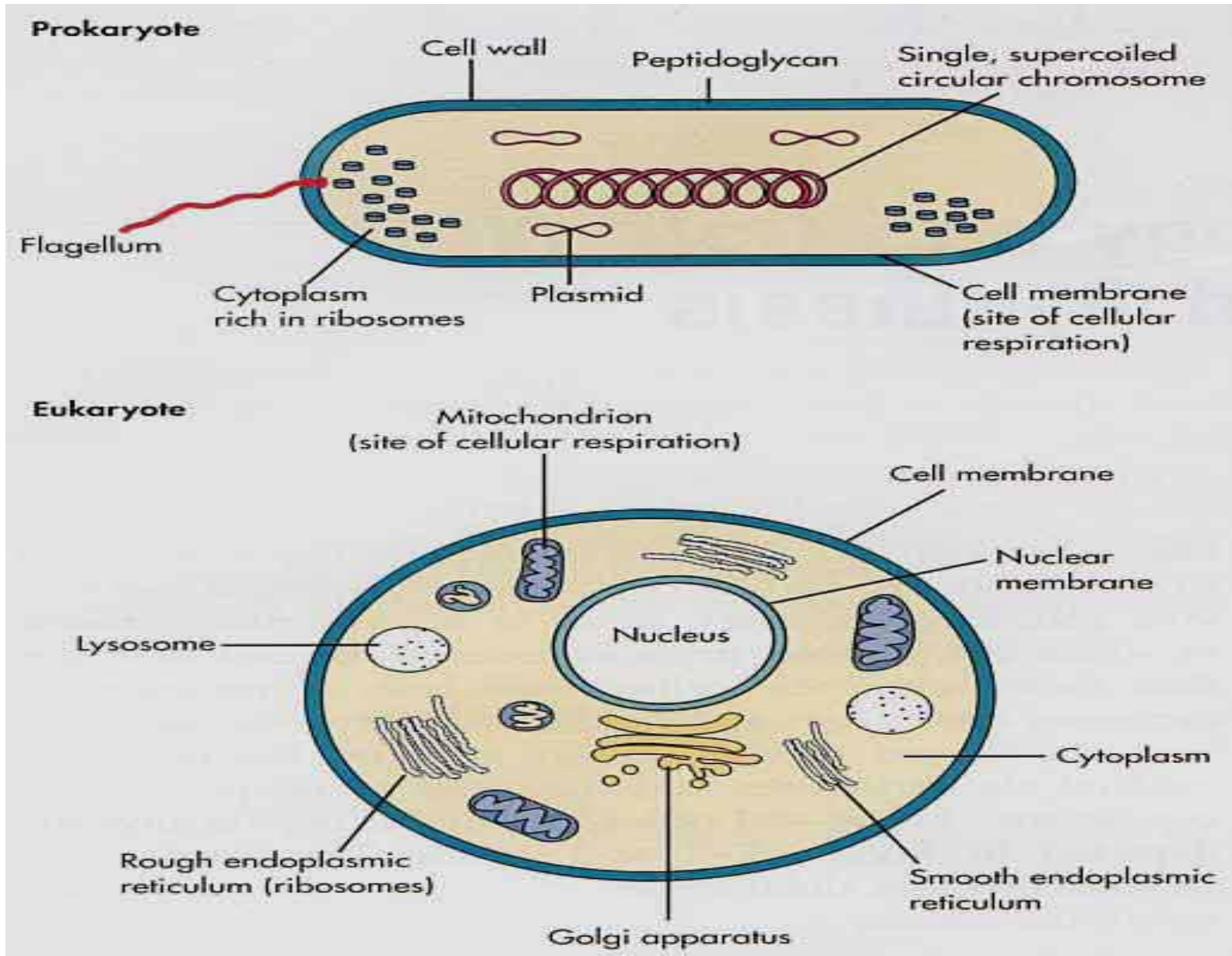
- Are more advanced life forms
- Possess a proper membrane bound nucleus (true nucleus), which contain chromosomes and contain organelles such as mitochondria, golgi apparatus, lysosomes and ribosomes
- Replicate by mitosis eg; Fungi, protozoa, plants and animals.

- Eukaryotes are grouped into the kingdom protista where as prokaryotes into kingdom monera
- Viruses fall into neither category

Comparison of eukaryotic and prokaryotic cells

Property	Prokaryotic	Eukaryotic
▪ True membrane bound nucleus	Absent	Present
▪ Number of chromosomes	One *	More than one
▪ Nucleolus	Absent	Present
▪ Replication	By binary fission	By mitosis
▪ Membrane bound organelles	Absent	Present
▪ Ribosomes	70S	80S
▪ Cell wall	Chemically complex with peptidoglycan	Chemically simple & lacking peptidoglycan
▪ Size	usually $\leq 5 \mu\text{m}$ in dm	Usually $\geq 100 \mu\text{m}$ in dm
▪ Differentiation	Rudimentary	Tissues & organs

Prokaryotes and Eukaryotes



Microscopy and microorganisms

- Microscope is an instrument most characteristics of the microbiology laboratory. It enables us to see microorganisms and their structures which are invisible to the naked eye. Depending upon the principle on which magnification is based, microscopes are of two categories
 - ✓ **Light or optical microscope**
 - ✓ **Electron microscope**

Light microscopy: magnification is obtained by a system of optical lenses using light waves.

- Two types namely Simple and Compound Microscope

Simple Microscope consists of a single lens.

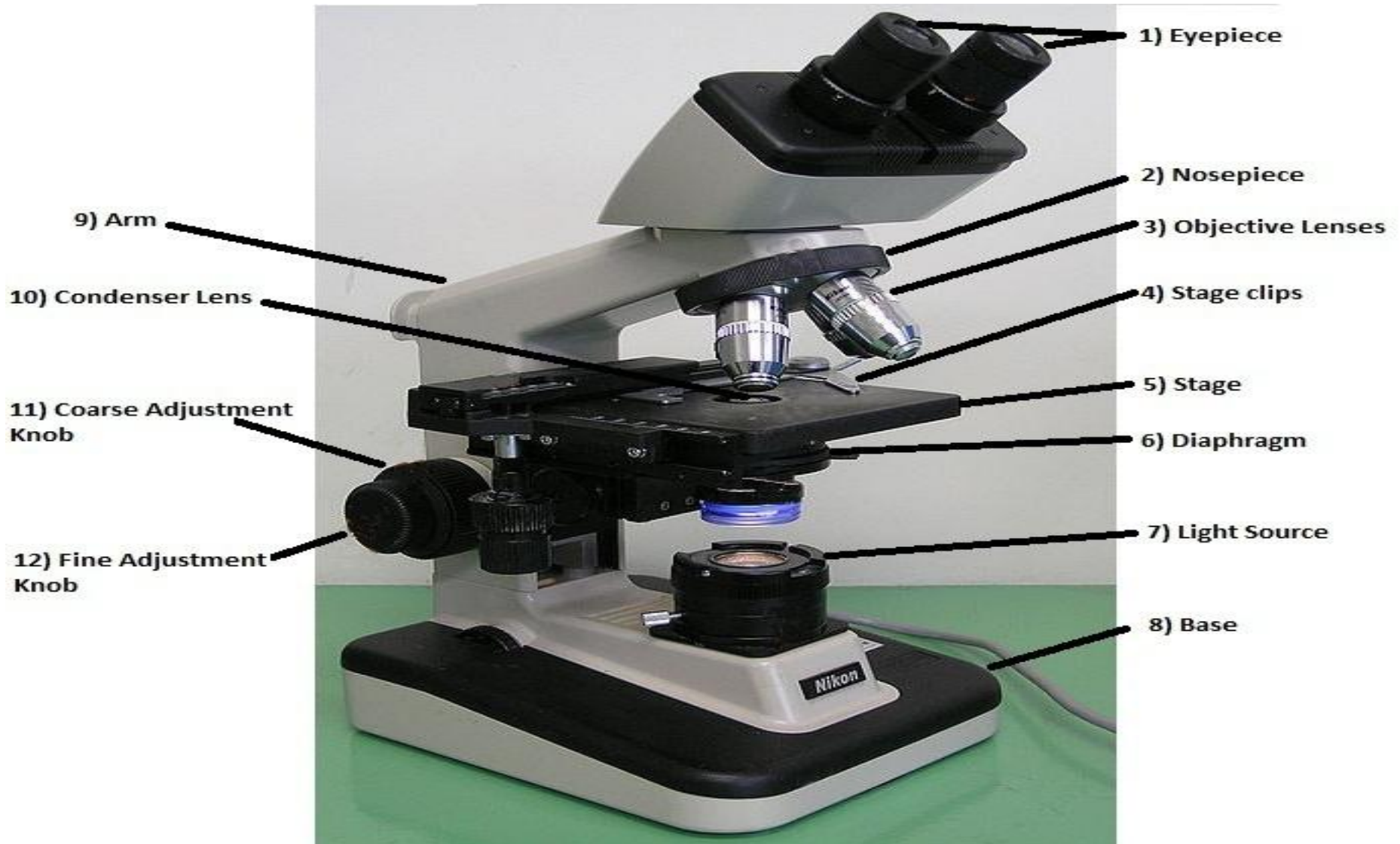
- A hand lens is an example of a simple Microscope.

Compound Microscope consists of two or more lenses in series. The image formed by the first lens is further magnified by another lens.

Simple microscope: consists of only a single lens. it is similar to a magnifying glass



Parts of a Compound Microscope



- Light microscopy, in which magnification is obtained by a system of optical lenses using light waves.

Types of light microscopes

Bright field microscope:

- ✓ Is the ordinary light microscope
- ✓ It is called a bright field because it forms a dark image against a brighter background

Dark- field microscope:

- The effect produce by the dark- field technique is that of a dark back ground against which objects are brilliantly illuminated

Phase-contrast microscope:

- ✓ Is valuable for studding living unstained cells and is widely used in applied and theoretical biological studies .

- ✓ It uses a conventional light microscope fitted with a phase contrast objective and a phase contrast condenser.

Fluorescence microscope:

- ✓ Exposes a specimen to ultraviolet, violet or blue light and forms an image of the object with the resulting fluorescent light.
- ✓ Usually the specimens have been stained with dye molecules, called fluorochromes

- To understand how a light microscope operates one must know the three principles of a light microscope; Magnification, Resolving power, Illumination

Magnification:

- The magnification of a compound microscope depends on ocular and objective lens systems.
- The total magnification of a microscope is equal to the product of the ocular lens and the objective lens magnifications.

Resolving power:

- The ability of a microscope to distinguish two adjacent points (particles) as distinct and separate

Resolving power can be increased either

- by reducing the wave length of the light or by increasing the numerical aperture.
- Since the spectrum of visible light is relatively narrow, the increase of the wave length of the light used is of limited value.

Illumination: The easily available source of illumination is ordinary day light but usually artificial light is used.

- The light from illumination source is refracted in to the sub stage condenser via the mirror located just below the condenser.

Electron microscope

- Uses a beam of electrons in place of light waves to produce the image. Specimens can be examined by either transmission or scanning EM
- EM has a practical resolution roughly 1,000 times better than the light microscope

- If electron illuminates the specimen, the microscope's resolution is enormously increased because the wavelength of the radiation is around 0.005 nm, approximately 100,000 times shorter than that of visible light.

Uses of different types of microscopes

Type of microscopy	Maximum useful magnification	Appearance of specimen	Useful application
Bright -field	1,000- 2,000	Specimens stained or unstained; bacteria generally stained and appear color of stain	For gross morphological features of bacteria,, yeasts, molds, algae and protozoa
Dark-field	1,000- 2,000	generally unstained; appear bright or “lighted” in an otherwise dark field	For microorganisms that exhibit some characteristic morphological feature in the living state and in fluid suspension eg. spirochetes
Fluorescence	1,000- 2,000	Bright and colored;; color of the fluorescent dye	Diagnostic techniques where fluorescent dye fixed to organism reveals the organism’s identity
Phase-contrast	1,000- 2,000	Varying degree of darkness	For examination of cellular structures in living cells of the larger microorganisms eg. Yeasts, algae, protozoa, and some bacteria
Electron	200,000- 400,000	Viewed on fluorescent screen	Examination of viruses and the ultrastructure of microbial cells

Morphology and Structure of Bacteria

- The word bacterium (plural, bacteria), is derived from the **Greek word** = *bakterion* = a small stick
- Bacteria vary in size as much as in shape (ranging in size from 0.2 μm -10 μm), **spiral organism** up to 100 μm , majority of the bacteria less than 5 μm , **Mycoplasma**=0.3 μm , **Nanobacteria** or **ultra microbacteria** = 0,2 to $\leq 0.05\mu\text{m}$ in dm. Some **spirochetes** occasionally reach 500 μm in length

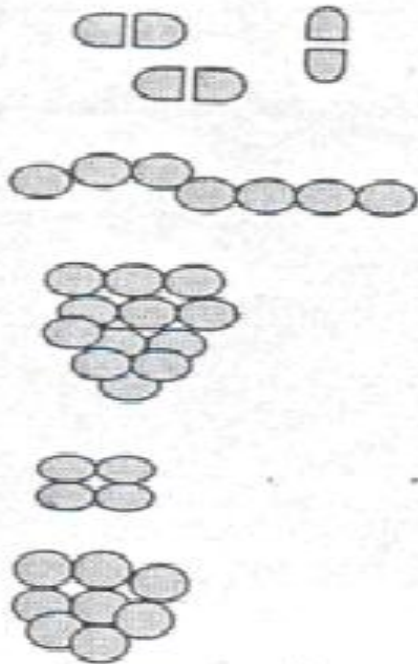
Generally, **bacterial cells** have three basic shapes

- **Round** (spherical): cocci/cocus
- **Rod-shaped** (bacilli/bacillus)
- **Spiral** with some varieties; spirilla/spirillum, spirochetes or curved rods

Cocci (Gr. Kokkos =berry) Cocci (coccus) are;

- Roughly spherical cells
- Can exist as individual cells
- Found in different arrangement depending upon their dividing planes

Cocci



Pairs (Diplococci) eg.
Neisseria

Streptococci

Staphylococci

Plates of four eg.
Gaffkya

Clubs of eight eg.
Sarcina

Rods or Bacillus (in Latin: bacillus =sticks)

- ✓ Bacilli differ considerably in their length-to-width ratio
- ✓ Coccobacilli being so short and wide that they resemble cocci
- ✓ The shape of the rod's varies between species and may be flat, rounded, cigar-shaped, or bifurcated

Rods



Coccobacilli eg. *Pasteurella*

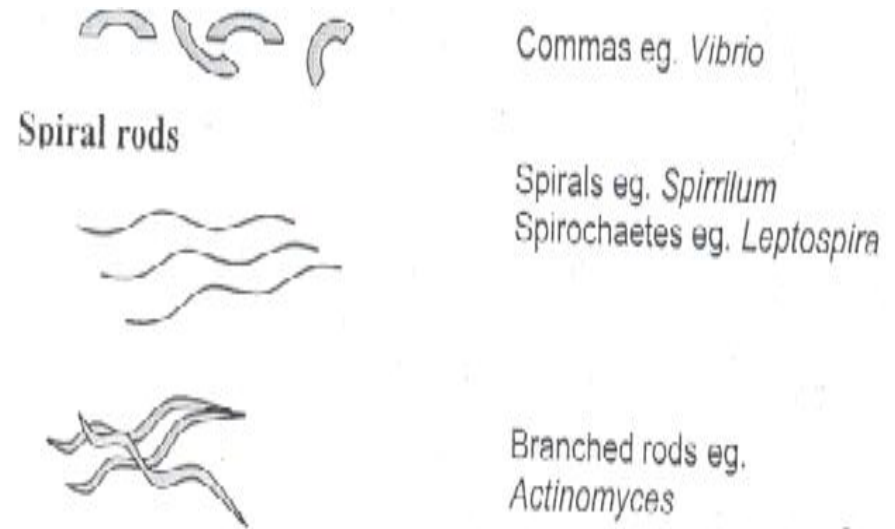


Chains eg. *Bacillus*

Spiral or curved:

Consists in the form of

- ✓ Vibrio
- ✓ Spirillum

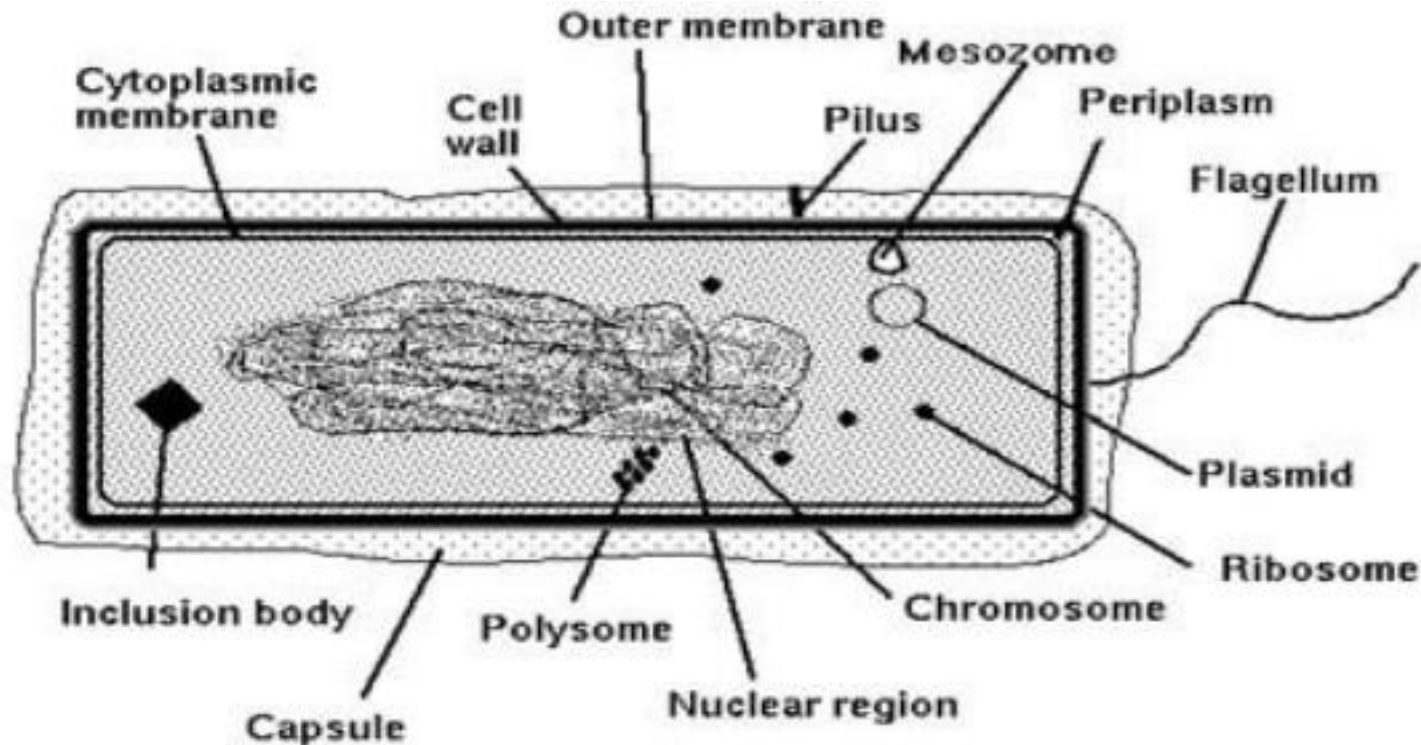


Pleomorphic bacteria

- ✓ Have variable shapes
 - ✓ Some bacteria show a degree of pleomorphism
 - ✓ example: *Mycoplasma*

Structure of the Bacterial cells

- Bacteria contain all the machinery required for growth and self- replication



Bacterial ultrastructures are:

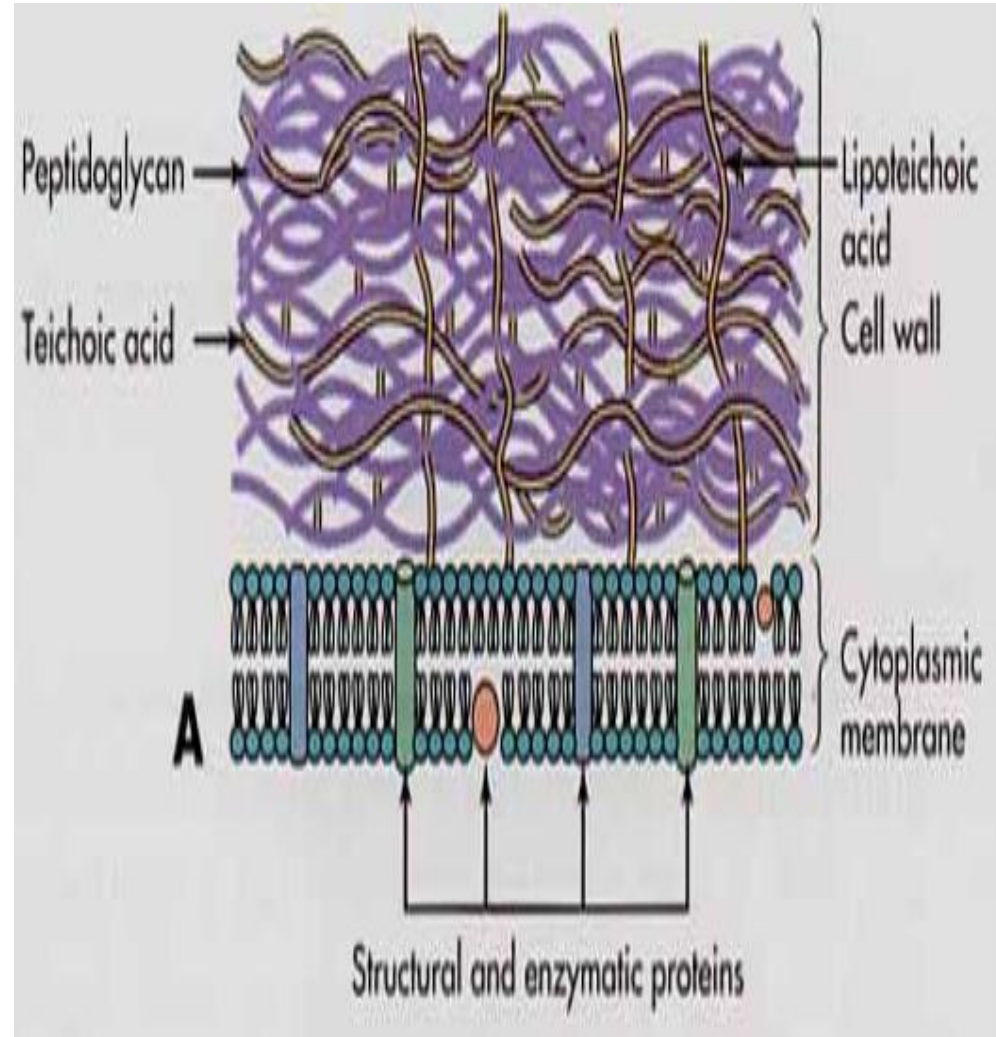
- ✓ Capsules
- ✓ Cell wall
- ✓ Cytoplasmic membrane
- ✓ Flagella
- ✓ Pili (Fimbriae)
- ✓ Spores
- ✓ Cytoplasm
- ✓ Ribosomes
- ✓ The nucleoid

Cell wall

- ✓ Protect the bacteria from chemical and physical action:
 - Determines the shape of the organisms
 - Bacteria are divided into two major groups, on the basis of color when stained by Gram method:
 - ✓ Gram positive
 - ✓ Gram negative

Gram positive

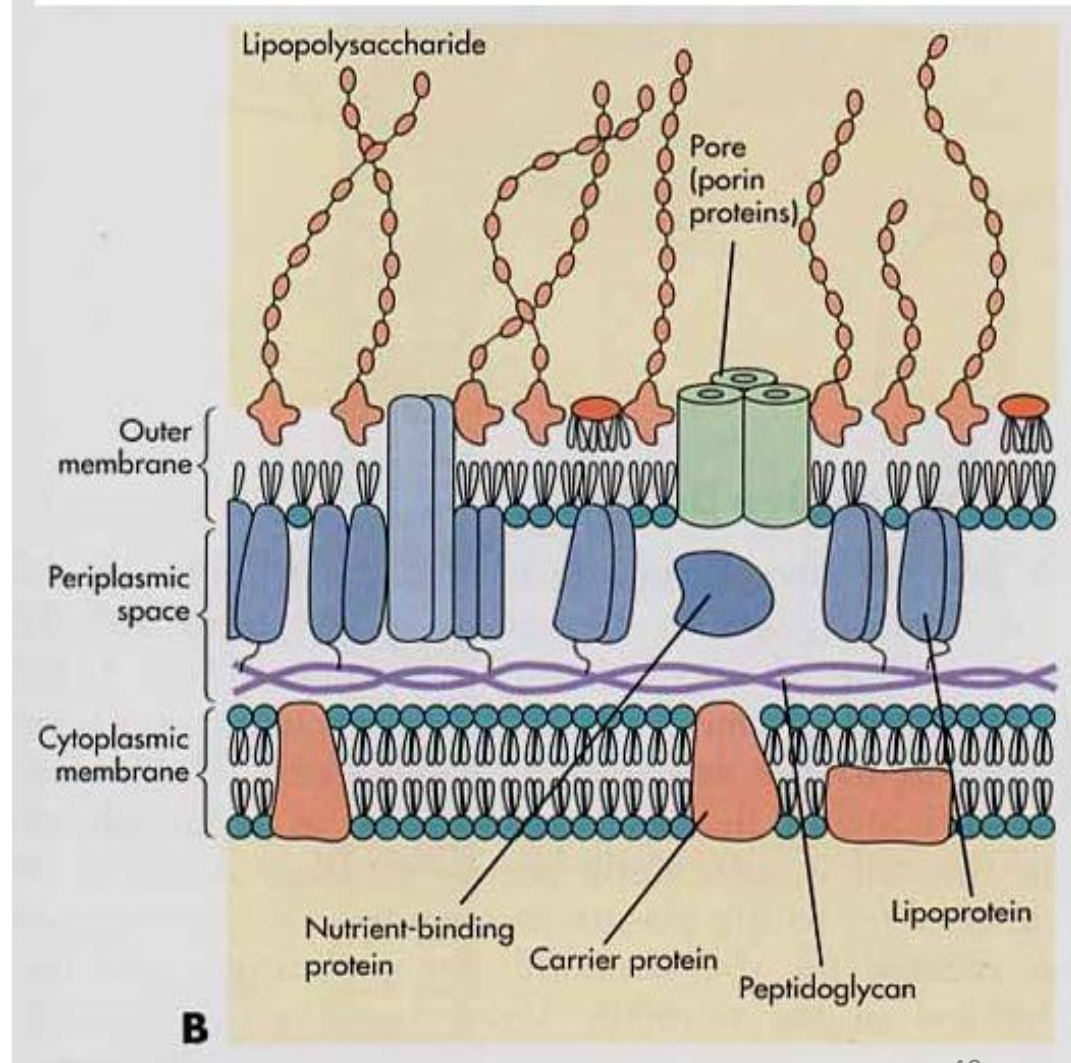
- Gram positive cell wall is relatively thick
- Composed mainly of peptidoglycan (Up to 40 layers)
- Include other components such as teichoic and lipoteichoic acid and polysaccharides



Gram -negative

Gram negative cell wall

- more complex
 - Contains two layers external to the cytoplasmic membrane
1. A thin peptidoglycan layer (only one or two layers)
 2. Outer membrane external to the peptidoglycan



Cytoplasmic membrane

- ✓ Composed of phospholipids and proteins
- ✓ It is permeable
- ✓ Located between the cell wall and the cytoplasm
 - Secret hydrolytic enzymes
 - Regulate cell division
 - Energy generation

Cytoplasm

- ✓ Aqueous fluid containing the nuclear material (DNA chromosomes ribosomes: protein synthesis)

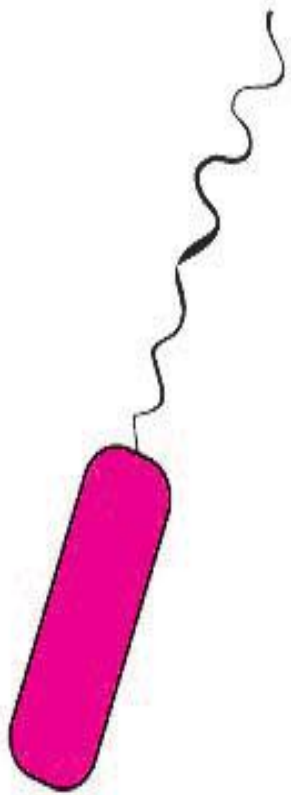
Capsule (Glycocalyx): mostly of water and 1-2% polysaccharide, polypeptide, or protein according to individual bacterial species. Its roles are

- ✓ Protection from adverse environmental condition
- ✓ Interfere with phagocytes in animal
- ✓ Facilitate the adherence of bacteria to animal tissue body

Flagella

- Attached to the bacterial cell wall
- Used for locomotion
- Composed of the protein flagellin
- Originate in the bacterial cytoplasm.
- Many Gram negative bacteria have flagella
- Flagella contain antigen (H antigen) which is used for identification and classification

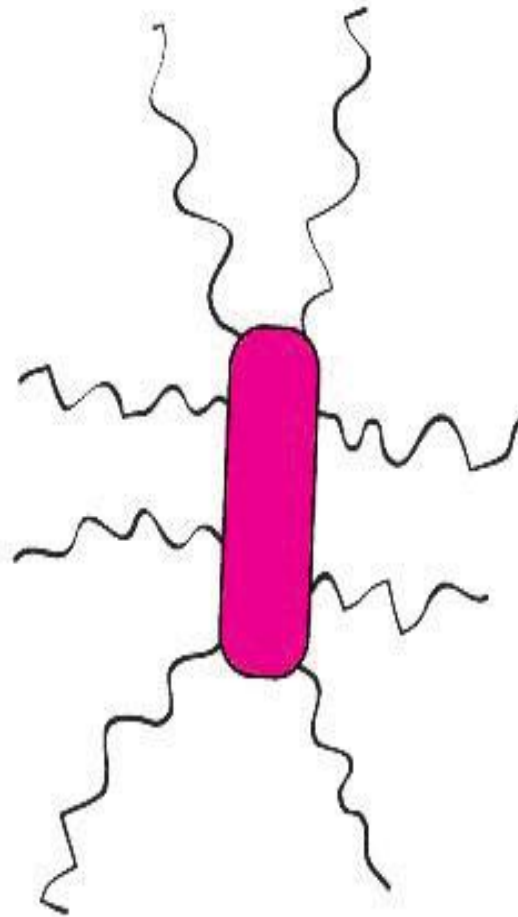
According to the position of flagella on the their surface, bacteria are divided in to 4



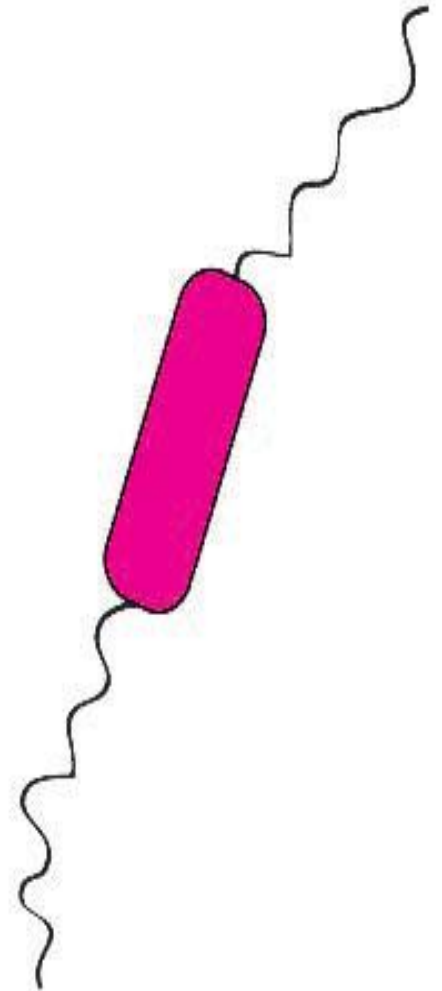
(a) Monotrichous
(polar)



(b) Lophotrichous



(c) Peritrichous



(d) Amphitrichous

Pili (Fimbriae):

- Hair like filaments appendages that extend from cell surface. They are composed of the protein pilin
- Found mainly on gram-negative organisms

Roles

- ✓ Promote the organism to adhere to mammalian cells
- ✓ Sex (conjugation) pilus promote the transfer of generic material (chromosomes) of bacterial during conjugation

Bacterial Spores

- Small, dehydrated metabolites produced during conditions that inhibit growth of cell division (nutritional depletion, in pH change, oxygen depletion)
- Formed inside the cell
- They are resistant to Heat, Dehydration, Radiation and Chemicals

Factors that play major role for the resistance

- ✓ Calcium dipicolinate in core
- ✓ Keratin spore coat
- ✓ New enzymes (dipicolinic acid synthetase, heat-resistant catalase)
- ✓ Increases or decreases in other enzymes.

Bacterial metabolism, growth and reproduction

Metabolic requirements

- Bacterial growth requires a source of energy and raw materials to build the protein structures and membranes that make up the structure and biochemical machines of the cell
- Bacteria must obtain or synthesize the amino acids, carbohydrates and lipids used as building block of the cell
- The minimum requirement for growth of bacteria need a source of Carbon and Nitrogen as energy source, Water and various ions are also necessary.

Bacteria can drive energy

- ✓ From the oxidation of metals ions
- ✓ By photosynthesis
- ✓ By metabolizing sugars, fats and proteins

Bacteria utilize energy for:

- ✓ The constriction of the physical parts of the cell (cell wall or membrane)
- ✓ Synthesis of enzymes, nucleic acid, polysaccharides, and other chemical component

Physico-chemical requirements for bacterial growth

- Bacteria grow if their environment is suitable. If the environment is not optimal; growth may occur at a lower rate or not at all or the bacteria may die.

Essential requirements for growth of bacteria include

- ✓ A Supply of suitable **nutrients**.
- ✓ A source of **energy** and **Water**
- ✓ An appropriate **temperature** and **pH**
- ✓ Appropriate levels (or the absence) of **oxygen**

Nutrients: Cell needs nutrients as raw materials for Growth, Maintenance and, Division.

- What ever the organism, cells need sources of, Carbon, Nitrogen, Phosphorus, Sulfur and other trace elements (materials) from which living mater is made

- Macro minerals are required in relatively large quantities
 - Carbon
 - Nitrogen
 - Hydrogen
 - Oxygen
 - Phosphorus
 - Sulfur
 - Calcium
 - Magnesium
 - Iron
- Micro minerals are required in relatively tiny quantities or trace amounts
 - Cobalt
 - Copper
 - Sodium
 - Manganese
 - Molybdenum
 - Zinc

Carbon:

- All microorganisms require carbon in, organic or inorganic forms
- Organic carbon forms the backbone of carbohydrates, lipids and proteins.

Bacteria can use

- Simple two carbon (acetate), Complex molecules (cellulose) or Monosaccharide (glucose)

Nitrogen

- It is the major component of proteins and nucleic acids
- Bacteria acquire Nitrogen from organic or inorganic sources

Inorganic sources of Nitrogen

- ✓ Ammonia, Nitrate and nitrite reduction ($\text{NO}_3^- / \text{NO}_2^- \rightarrow \text{NH}_3$)
- ✓ Gaseous N_2 (N_2 - fixation) $\text{N}_2 \rightarrow \text{NH}_3$

Organic sources of Nitrogen

- ✓ Proteins or their degradation products, In the process of N_2 assimilation: NH_3 formed from different N sources
- ✓ Glutamic acid + NH_3 = produce glutamine, which serves as a storehouse of N_2

Phosphorus:

- Sources of Phosphorus: inorganic phosphate (PO_4^-)
- Essential for the synthesis of important biomolecules like Nucleic acids and ATP (Adenosine triphosphate)

Sulfur:

Needed for the biosynthesis of the Amino acids like

- ✓ Cystine
- ✓ Methionine

Calcium;

- ✓ Acts as enzyme cofactor
- ✓ Contributes to heat resistance of spores

Potassium:

- Activates various enzymes

Magnesium:

- Serves a cofactor for many enzymes. It also stabilizes membranes and ribosomes and nucleic acids

Iron:

- It is important component of the cytochromes (electron carries in oxidation reduction reactions) system

Temperature requirements for bacteria

- ❖ For a given type of bacterium, growth occurs most rapidly at a particular temperature (optimum temperature). There are also maximum and minimum temperatures beyond which growth will not occur.
- Based on temperature requirements: bacteria grouped into
 - ✓ Psychrophiles
 - ✓ Mesophiles
 - ✓ Thermophiles

Psychrophiles (Cold- loving)

- Grow between -10°C and 20°C
- Psychrophilic bacteria and fungi are major factors in the spoilage of refrigerated foods
- Found in cold water, mud and soils such as in the polar region
- Examples: *Pseudomonas*, *Flavobacterium* *alcaligenes*, *Polaromonas vaculata*

- **Mesophiles:** are microorganisms with growth temperature optima 20°C - 45°C
 - Example: most human and veterinary pathogens
- **Thermophiles:** They can grow between 45°C - 70°C
 - ✓ Possess heat stable enzymes and
 - ✓ Protein synthesis system able to function at high temperature

Hyperthermophilic bacteria

- Have growth optima between 80 –113 °C
- ✓ Do not involve in infectious diseases
- ✓ They are found in
 - Volcanic areas
 - Compost heaps
 - Hot springs

pH requirements of bacteria

- Based on pH requirements bacteria are classified in to
 1. **Neutrophiles** prefer the pH range of 5.5 to
 2. **Acidophiles** have their growth optimum between pH 0 and 5.5.
 3. **Alkalophiles** prefer the pH range of 8.5 to 11.5
- Most bacteria grow best at or near pH 7.0 (neutral)

Oxygen requirements of bacteria

- Some bacteria need oxygen for their growth.
 - Others do not need oxygen for their growth
 - others can grow regardless of the presence or absence of oxygen.
- ❖ Based on the requirements of O_2 bacteria can be grouped
- a. Aerobes**
 - b. Strict or obligate anaerobes**
 - c. Facultative anaerobes**
 - d. Microaerophilic bacteria**
 - e. Aerotolerant**

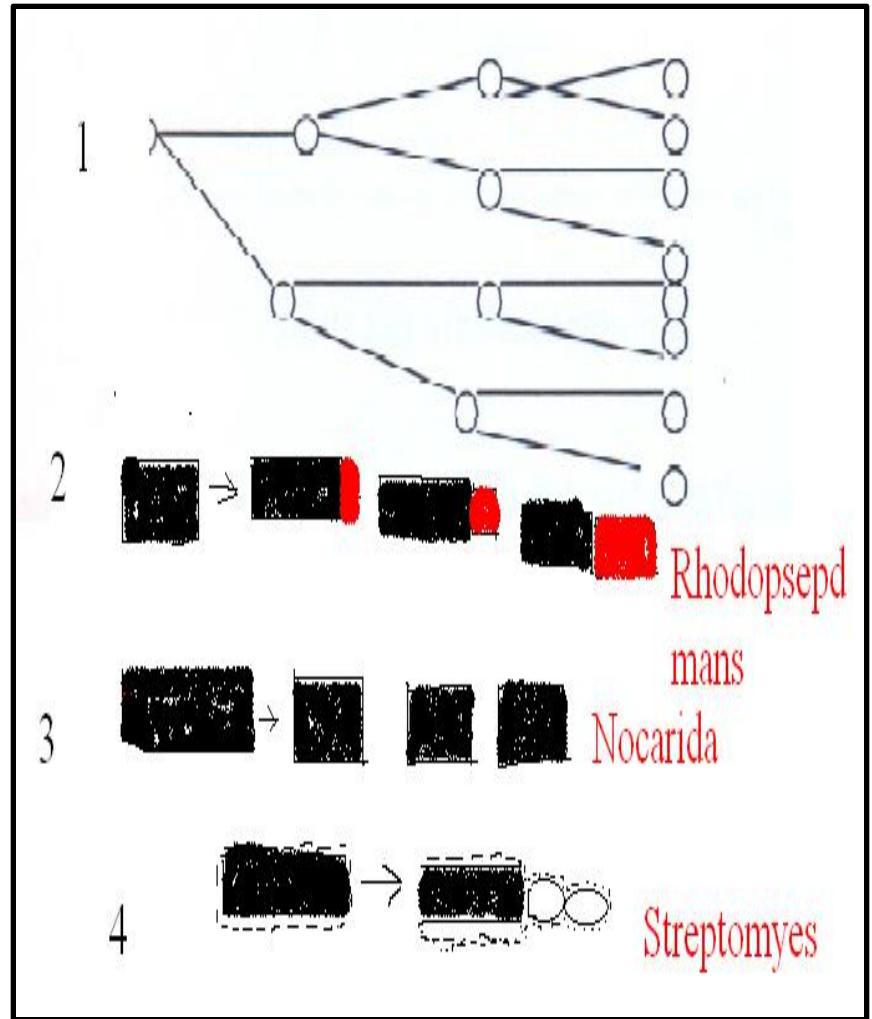
- **Aerobes:** organisms that growth in the presence of oxygen
- **Strict or obligate anaerobes:** grow only when oxygen is absent
- Bacteria which normally grow in the presence of oxygen but which can still grow under anaerobic conditions (ie. In the absence of oxygen) are called **facultative anaerobes**
- Similarly those which normally grow anaerobically but which can grow in the presence of oxygen are called **facultative aerobes**

- **Microaerophilic bacteria:** generally grow best when the concentration of oxygen is /usually much/ lower than the normal atmospheric level
- **Aerotolerant anaerobes:** Ignore oxygen and grow equally well whether it is present or not

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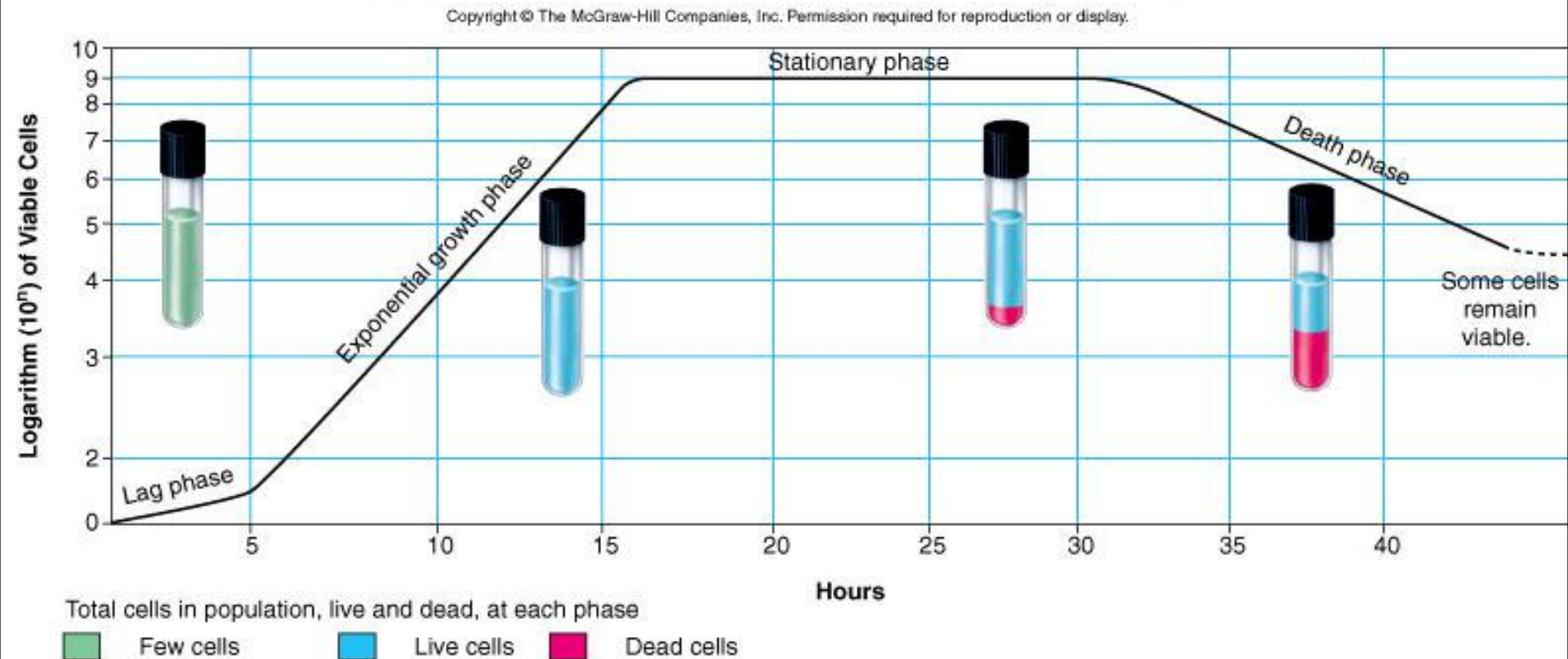
Group	Environment		O ₂ Effect
	Aerobic	Anaerobic	
Obligate Aerobe	Growth	No growth	Required (utilized for aerobic respiration) 1atm(21%)
Microaerophile	Growth if level not too high	No growth	Required but at levels below 0.2 atm
Obligate Anaerobe	No growth	Growth	Toxic
Facultative (An)aerobe	Growth	Growth	Not required for growth but utilized when available
Aerotolerant Anaerobe	Growth	Growth	Not required and not utilized

- Reproduction of bacteria take place by
 - ✓ binary fission: most common
- Alternative means
 - ✓ Budding
 - ✓ Conidiospores (filamentous bacteria)
 - ✓ Fragmentation



Bacterial growth phases (curves)

- The growth curves of bacteria are characterized by distinct stages from the time it is introduced into a medium until it ceases growth. These are **Lag**, **log (exponential)**, **stationary**, **decline** or **death**.



Nomenclature and classification of Bacteria

- Taxonomy is an area of biological science which comprises three distinct, but highly interrelated disciplines that include classification, nomenclature and identification.
- Applied to all-living entities, taxonomy provides a consistent means to classify, name and identify organisms. The common language that taxonomy provides minimizes the confusion about names of Mos.

- Classification is the organization of organisms that share similar morphologic, physiologic and genetic traits into specific groups or taxa.
- Nomenclature, the naming of microorganisms according to established rules and guidelines provide the accepted labels by which organisms are universally recognized.

- So the major aim of taxonomy is to set up classification of microorganisms
- ✓ This classification can then be used to identify individual bacterial species or strains (identification) and name them (nomenclature)

Rules of bacteriological nomenclature

- ✓ Bacterial nomenclature is the assignment of names to taxonomic groups according to international rules which are governed by 'Bacteriological code' including:
 - Particular species name has only one name understood by many scientists

- The name does not need to be descriptive one:- in fact descriptive names are prone to be misleading

For example: the name *Staphylococcus aureus* is descriptive of the golden *Staphylococcus*, however, it is now recognized that this organism may become non pigmented or white

- The first name (Genus name) of the organism should be a proper noun and always begins with a capital letter
- The second name begins with small letter and designates the species name
- The whole binomial name must be Latinized and italicized or underlined (Example: *Staphylococcus aureus*)

- ✓ The taxonomic categories into which the bacteria can be ranked from highest to lowest,

Kingdom, Phylum, Class, Order, Family, Genus, Species

- ✓ The names of each level ends with specific suffix. Eg.
Family- **aceae** (Enterobacteriaceae) Order- **ales**
(Enterobacterales), Class-**ia** (Gammaproteobacteria)

Genus: Escherichia

Species: *Escherichia coli*

Characteristics used in bacterial identification and classification

Bacteria are classified by using any of the characteristics under the following general heads

I. DNA test: comparison of DNA content and sequence between strain. These tests are the most definitive methods for separating organisms into different groups.

- DNA is analyzed by measuring the guanine- cytosine (G-C) content or the amount of DNA homology between strains.

II. Colony morphology: The shape, texture and color of colonies of microorganisms growing on solid agar plates are used for differentiation when ever practical. eg. *Staphylococcus aureus* is so named as colonies are of a yellow color; aureus comes from Latin for gold

III. Reaction to stains: Gram staining classified bacteria into Gram positive and Gram negative

- Stains may also be used to show other morphological features, which may used for classification, such as
- Spores (Malachite green stain)
 - Unusual cell wall (acid fast stain)
 - Capsules (Indian ink stain)
 - Intracellular lipids (Sudan black)
 - Flagella (flagellar stain)

IV. Growth Characteristics:

- Relation to temperature (*Campylobacter jejuni*, grows well at 42° C; another, *Yersinia enterocolitica*, grows better than most other bacteria at 4° C)
- Relation to pH,
- Oxygen requirements of an isolates are useful tests for the identification (aerobically, anaerobically, facultatively (i.e., in either the presence or absence of oxygen))

V. Biochemical tests: Used to measure various aspects of bacterial metabolism such as ;-

- What Carbon and nitrogen sources the bacteria can use ...like sugars
- The end product of their metabolic process, such as acetoin, acids, gases
- What enzymes the bacteria produce,
- Resistance to antibiotics

VI. Antigenecity:

- Cellular components such as the O-side chains of lipopolysaccharide (LPS) or capsule are frequently used to distinguish between strains of one species
- The antigenic relationships of bacteria are detected by the use of serological tests agglutination and precipitation

❖ Bacterial Genetics

- **Bacterial genetics** describes the characteristics of bacterial DNA (plasmids) and the way how this genetic material transfer among bacterial population
- **Bacterial genetic material**
 - ✓ is the total collection of **genes**
 - ✓ Since bacteria have only one chromosome; alteration of a gene (Mutation) will have a more obvious effect on the cell

- **bacteria transmit characteristics to their progeny(information from generation to generation with great accuracy)**
- **. However, in addition to the inheritance of characters, there is also variability to change expressed in progeny. Genetic variation in bacteria occurs due to**
 - ✓ **Mutation**
 - ✓ **Gene transfer (recombination**

- **Mutation**

- Mutation is a stable and heritable change in the base sequence or number of nucleotides in a DNA molecule (hereditary material).
- The change :Heritable & irreversible, unless there is back mutation to the original sequence.
 - ✓ Some mutations may be lethal, while others may help the organism to survive in its
- Environment play an important role in generating genetic diversity.
- Mutation may be arise
 - ✓ spontaneously
 - ✓ induced

- **Spontaneous mutation:** is mutation occurring during DNA replication.
- This is an intrinsic error in DNA replication.
- The rate of spontaneous mutation in bacteria is 10^{-6} to 10^{-10} per cell division per gene
- **Induced mutations:** more mutation induced or inflicted by mutagens (agents that cause mutation).
- ✓ Mutagens may be chemical or physical agents like ultraviolet radiation and X-rays.
-

Cond,

- **Genetic recombination Gene transfer):**
The exchange of DNA between cells allows the exchange of genes and characteristics between cells, thus producing new strains of bacteria
- Gene transfer can be performed by
 - ✓ Transformation
 - ✓ Conjugation
 - ✓ Transduction
 - ✓ In each of these processes there is a transfer of genes from the donor to recipient DNA

- **Transformation**

- take up fragments of naked DNA and incorporate them into their genomes.

- **Conjugation**

- It is a process by which DNA is passed directly by cell-to-cell contact during the mating of the bacteria

- **Transduction**

- ✓ Transduction is a process of gene transfer from one bacterium to another by bacterial viruses

Bacterial Pathogenesis

- The animal body is a collection of **environmental** niches /place/ that provide the **warmth, moisture** and **food** necessary for **growth** of bacteria
- Many of the **mechanism** that bacteria use to maintain their niche and the by products of bacteria growth (eg. Acid, gas), cause damage and problems for the **animal host**.

- **A Pathogen:** is a microorganism that is able to cause disease in an organism (a plant, animal or insect).
- **Pathogenesity:** is the ability to produce disease in a host organism.
- Microbes express their pathogenesity by means of their **virulence**, a term which refers to the degree of pathogenesity of the microbe. The degree of virulence is related directly to the ability of the organism to cause disease despite host resistance mechanisms;

- Although many bacteria cause disease by directly destroying tissue and release toxins, which are then disseminated by the blood to cause system wide problem, not all bacteria cause disease.

Con't..

So, depending on their nature the animal body may interact with

- Normal bacterial flora
- Opportunistic bacteria and
- Virulent bacteria

- **Normal bacterial flora:** The animal body is colonized with numerous microbes /normal flora/, many of which serve important functions for their host such as
 - ✓ Aiding in the digestion of food
 - ✓ Producing vitamins (eg. Vitamin k) and
 - ✓ Protecting the host from **colonization** with **pathogenic** microbes.

NB: They can cause disease if they enter normally sterile sites of the body

Con't..

- **Opportunistic bacteria:** cause disease only in animal with conditions that enhance their susceptibility (Example: when defense systems of the host become debilitated.) They may live as commensally in the body.
- **Virulent bacteria:** are pathogenic and have the ability to invade their hosts and produce disease. They have mechanisms that promote their growth in the host at the expense of the host's tissue or organ.

- Virulence-associated factors may be defined as all factors that are essential for expressing Pathogenesicity. The determinants of virulence of a pathogen are any of its genetic or biochemical or structural features that enable it to produce disease in a host.

Examples of virulence factors for bacteria

- Virulence factors help bacteria to (1) invade the host, (2) cause disease, and (3) evade host defenses. The following are types of virulence factors:
- **Adherence Factors:** Many pathogenic bacteria colonize mucosal sites by using *pili* (fimbriae) to adhere (attach) to cells.
- **Invasion Factors:** Surface components that allow the bacterium to invade host cells (e.g. flagella)

- **Capsules:** Many pathogenic bacteria possess polysaccharide capsules that protect them from opsonization and phagocytosis.
- **Endotoxins:** It is comprised of toxic lipopolysaccharide components of the outer membrane of Gram-negative bacteria. It is the class of toxic substances released after lysis of bacteria.

- **Exotoxins:** Exotoxins include several types of protein toxins and enzymes produced and/or secreted from live pathogenic bacteria. Exotoxins are produced by some members of both Gram-positive and Gram-negative genera. Major categories include cytotoxins, neurotoxins, and enterotoxins.

N.B. Whether a host will develop disease is, however, not just determined by the pathogenic potential (virulence) of the bacterium, but also by host factors.

- So, the pathogenesis of the bacteria is a multifactorial process which depends on the immune status of the host, the nature of the bacterial species or strain, and the number of organisms in the exposure.

Con't..

Some underlying mechanisms of bacterial pathogenesis

1. The ability to invade tissues: Invasiveness, which encompasses mechanisms for colonization (adherence and initial multiplication), ability to bypass or overcome host defense mechanisms, and the production of extracellular substances which facilitate invasion.
2. The ability to produce toxins: Toxigenesis. Bacteria produce two types of toxins called exotoxins and endotoxins.

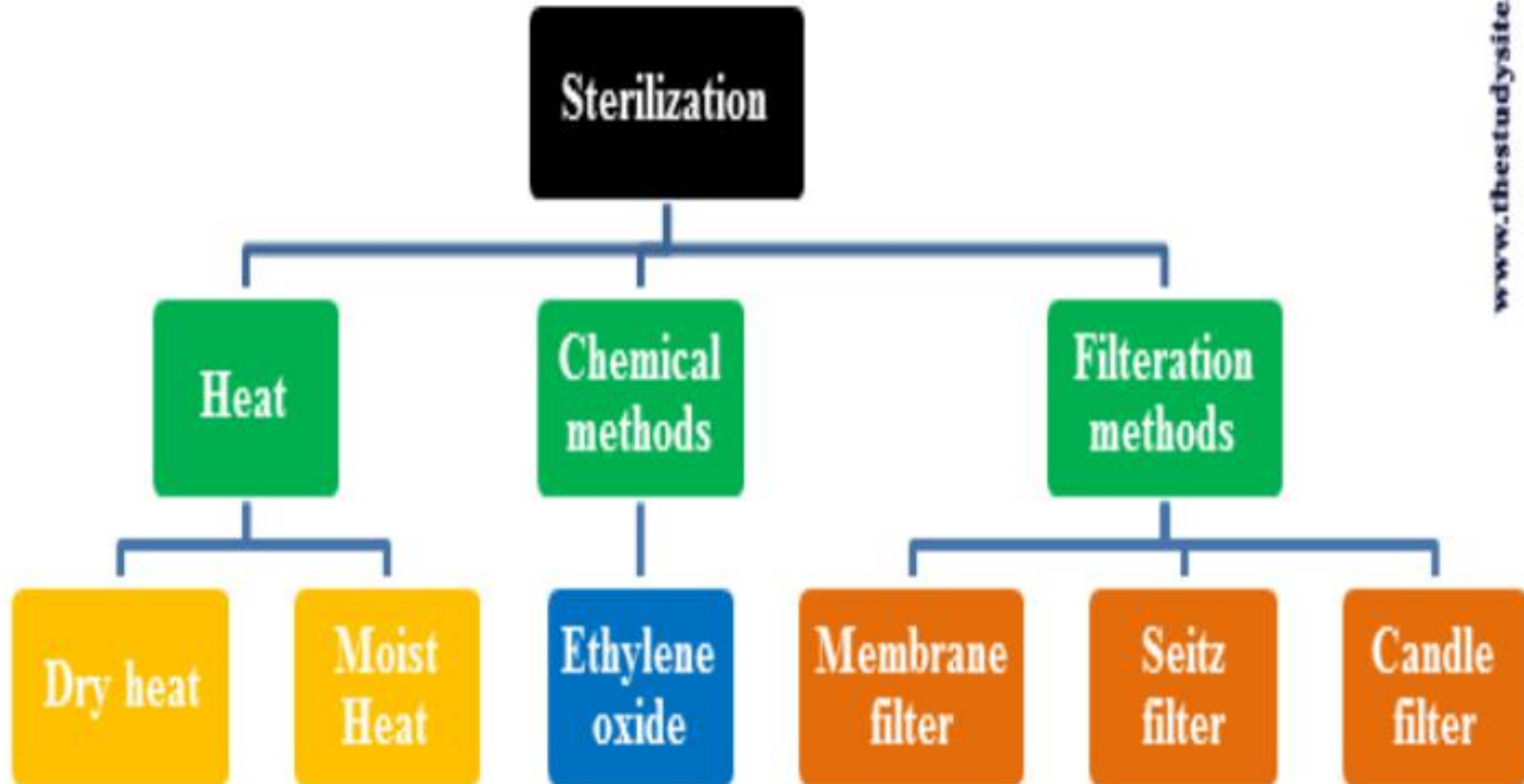
Sterilization and disinfection

- Bacteria are present on the surface of all laboratory apparatus, in the dust, upon the hands and are generally found every where.
- They are the source of contamination, infection, and decay. Hence it is necessary to remove them from materials and areas.

Sterilization

- ✓ Means total destruction of all microbes including the more resilient forms such as bacterial spores, mycobacterium, non-enveloped viruses and fungi
- ✓ Sterilization can be achieved by
 - Heat
 - Chemical
 - Filtration methods

Methods of sterilization



Heat sterilization

- Most common method of sterilization.
- The heat kills the microbes in the substance.
- The amount of heat and duration of heating are the factors that affect extent of sterilization. two types based on the type of heat used
 - ✓ Moist heat method
 - ✓ Dry heat method

Moist heat methods of sterilization:

- ✓ Boiling
- ✓ Pasteurization
- ✓ Use of pressurized steam (Autoclaving)

- Boiling is preferred for metallic devices like surgical scissors, scalpels, needles etc.



B. Pasteurization is a process of heating the milk at a temperature of 63 °C or 72°C for 30 min and 15 sec, respectively

C. Steam (autoclaving): Substances are subjected to sterilization in an autoclave.

- Three major factors required for effective autoclave
 - ✓ Pressure
 - ✓ Temperature
 - ✓ Time

Pressure	Temperature	Time (Min.)
15 psi	121°C	15
20 psi	126°C	10
20 psi	134°C	3

Dry-heat sterilization

- Requires a higher temperature than moist heat and a longer exposure time.
- More convenient for heat-stable, non-aqueous materials that cannot be sterilized by steam (strong glasses like petridish and tubes)

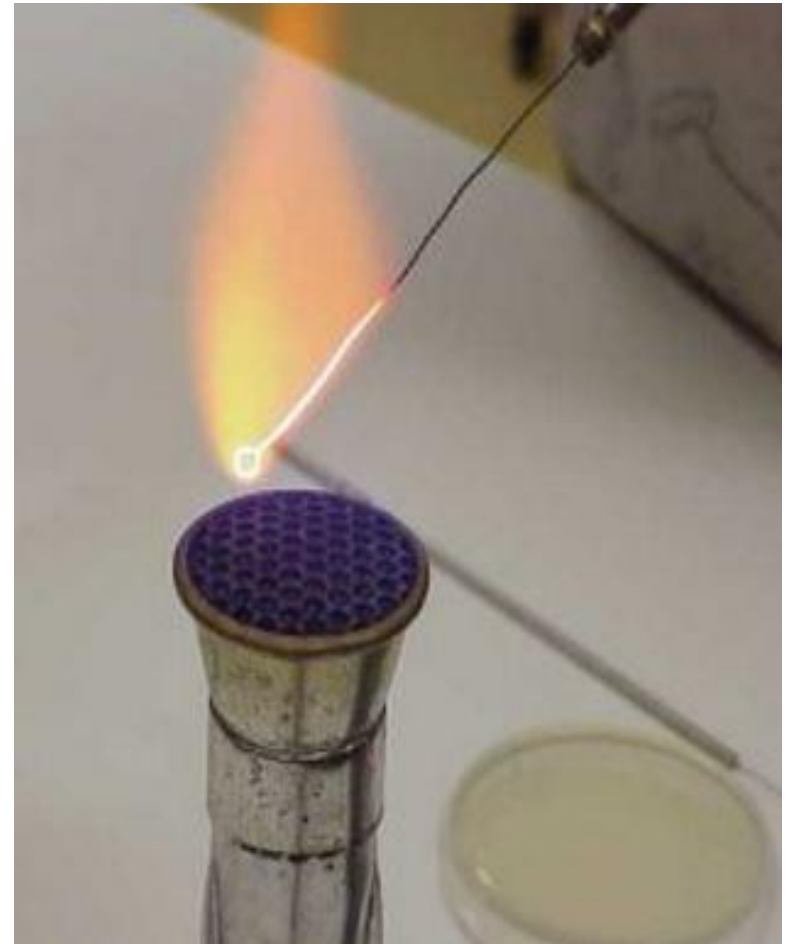
<u>Temperature °C</u>	<u>time in min</u>
• 160	180
• 170	60
• 180	30

This method includes techniques like

- ✓ Red heat
- ✓ Flaming
- ✓ Incineration
- ✓ Hot air oven
- ✓ Radiation sterilization

Red heat

- It is used to sterilize metallic objects (needle, scalpels, scissors etc) by holding them in flame and heated red hot. The fire burns the microbes and other dust on the instrument



Flaming

- The material is passed over flame without allowing it to become red hot. used for sterilizing scalpel, mouth of culture tubes, glass slides etc.



- **Incineration:** for items like as bandages, paper dishes, and sputum caps
- **Hot air oven:** (oven baking) suitable for drying materials like powder, metal devices, glassware



Radiation method:

- Involves exposing the packed materials to radiation for sterilization
- ✓ Two types of radiation
- Non ionic radiations: Safe to the operator , like UV radiation
- Ionizing radiations: powerful radiation, harmful the operator needs protect himself from exposure by dressing special cloth. Eg. X-rays, gama-rays. Etc

Chemical methods of sterilization

- Articles are subjected to sterilization by using toxic gases
- Gas penetrates quickly into the material
- But the chances of explosion and cost factors are to be considered
- The commonly used gas is ethylene oxide with combination of CO_2 . CO_2 is added to minimize the chances of an explosion

Filtration method of sterilization:

- Liquids are filtered through bacterial filters to remove any microbes present
- Useful to sterilize heat sensitive objects.

Disinfection: is a process, which involves use of physical procedures or chemical agents (disinfectants) to destroy most microbial forms

Disinfectants are subdivided into

- ✓ High Level
- ✓ Intermediate level
- ✓ Low-level agents
- ✓ applied on inanimate objects

- Examples of disinfectants include Glutaraldehyde, hydrogen peroxide, peracetic acid, chlorine dioxide, and other chlorine compounds

Antisepsis

- Is the use of chemical agents on skin or other living tissues to inhibit or eliminate microbes; no sporicidal action is implied. Antiseptics include
 - ✓ Alcohols
 - ✓ Iodophors .
 - ✓ Chlorhexidine
 - ✓ Triclosan etc

Culture media

- Media (Medium S.): is a substance used to provide nutrients for the growth and multiplication of microorganisms. It gives artificial environment, simulating natural condition necessary for growth of bacteria
- Used for
 - ✓ Isolation
 - ✓ Identification
 - ✓ antibiotic sensitivity

Culture media should contain

- ✓ Energy source
- ✓ Carbon source
- ✓ Nitrogen source
- ✓ Salts
- ✓ Satisfactory PH
- ✓ Adequate oxidation-reduction potential and
- ✓ Growth factor

Based on their consistency culture media are classified into:

- ✓ Fluid laboratory media
- ✓ Semisolid laboratory media
- ✓ Solid laboratory media

Liquid media

- ✓ Used as enrichment media before plating on solid media
- Bacteria grow very well in fluid media in 3 to 4 hours
- These are not suitable to study colony types

Types :- (Broth, Peptone, Yeast extract)

Solid



Liquid



semi-solid



Culture Media

Solid Media

- Used to study colonies of individual bacteria.
- Essential for isolation of organism in pure form

Types

- ✓ **Agar:** It is complex polysaccharide obtained from sea weeds (Algae geledium species)
- ✓ **Gelatin:** protein prepared by hydrolysis of collagen with boiling water

Based on the ingredients that lab media contain
classified : Defined or synthetic media and Complex
Media

Defined or synthetic laboratory medium

- ✓ The exact amount of each component is known
- ✓ Used widely in research, as it is often desirable to know what the experimental microorganism is metabolizing

Complex Media

- ✓ Contain some ingredient of unknown chemical composition.
- ✓ Examples: Meat extract, yeast extract, MacConkey agar

Based on the purpose in which laboratory media are used can be divided in to the following categories

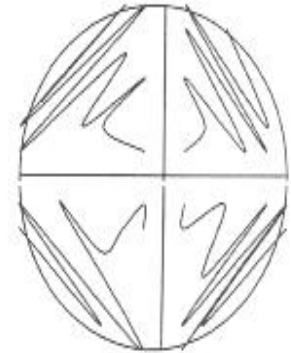
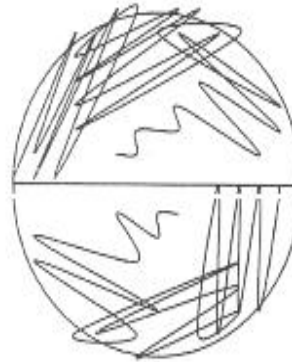
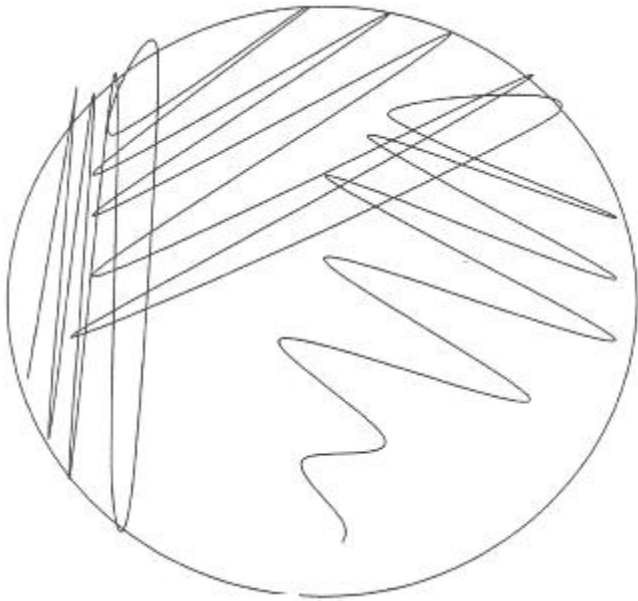
- **General purpose media:** support the growth of many MOs e.g. Tryptic soya broth, nutrient agar
- **Enriched media:** support the grow of fastidious bacteria (require many growth factors) blood ,serum, etc
- **Selective media:** made selective for the growth of a particular bacterium. They contain substance that prevents the growth of unwanted bacterial species. Eg. MacConkey agar, Endo agar , Eosin methylene blue agar

- **Indicator media:** Designed to give a presumptive identification of bacterial colonies and contain fermentable sugars plus a pH indicator that gives a color change in the media. Example MacConkey media
- **Differential media:** distinguish between different groups of bacteria based on their biological characteristics. e.g. Blood agar

Inoculation of Culture Media:

- The processes of introducing a tiny sample into a container of medium.
- There are different types of inoculation (planting) methods on to petridish these are:
 - A. Quadrant plating method: using the whole plate
 - B. Half plating: using half of the plate
 - C. Quarter plating: using quarter r of the plate

Different bacterial inoculation methods



There are also different techniques of inoculation some of them are

1. Streak culture: -

- The objective of plate Streaking is to obtain isolated bacterial colonies.

2. Spread Plate: is used to separate microorganisms contained within a small sample volume, which is spread over the surface of agar plate,

3. Pour plate culture

- ✓ Appropriate dilution of inoculums mixed with melted agar is poured or dispense in to Petri-dish. gives viable bacteria count in a suspension.

Measurement of bacterial growth: can be done by direct or indirect methods

- ✓ **Cell mass:** Directly weighing or by measurement of cell nitrogen or indirectly by turbidity
- ✓ **Cell activity:** indirectly by relating the degree of biochemical activity to the magnitude of the bacterial population
- ✓ **Cell count:** directly by microscopy or using an electric particle counter. Bacteria can be counted easily and accurately with the Petroff-Hausser counting chamber
- ✓ **Colony count:** The most frequently used method of counting bacteria is plate count method

Staining of bacteria

- ✓ Living microorganisms can be directly examined with the light microscope.
- ✓ They often must be fixed and stained to increase visibility of specific morphological features, and preserve them for future study.
- ✓ Before we can begin the staining procedure, the cells have to be mounted (smear) and fixed onto a glass slide.

Staining techniques

✓ Simple and differential staining techniques

Simple: using only one dyes,

- Cover the fixed smear with anyone of the following dyes: (Gentian violet ,Crystal violet, Safranin, Methylene blue, Basic fuchSION)
- After 30-60 seconds the slide is washed under the water tap and the smear is gently blotted dry.
- Size, shape and arrangement of bacteria are appreciated

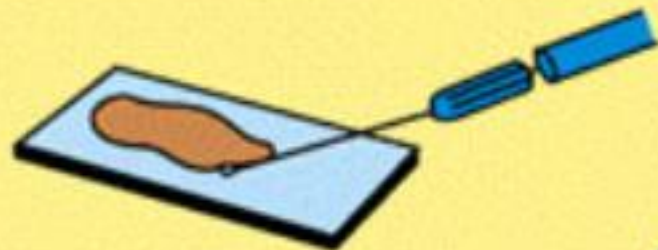
Differential Staining Techniques

- In microbiology, differential staining techniques are used more often than simple stains.
- Require more than one stain and several steps, are referred to as such because they permit the differentiation of cell types or cell structures.
- The most important of these is the Gram stain. Other differential staining methods include the endospore stain (to identify endospore-forming bacteria) and the acid-fast stain

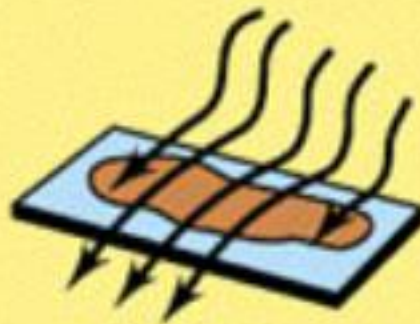
Gram staining procedure

Fixation

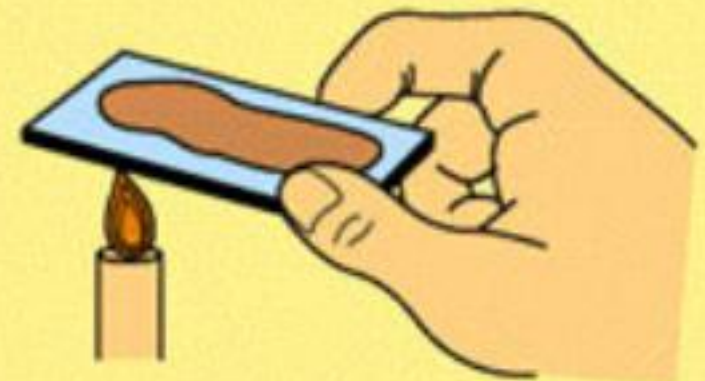
- Make a thin film of the material on a clean glass slide
Air dry, fix the slide by passing it several times through a flame (the slide should not become too hot to touch)



Spread culture in thin film over slide



Dry in air

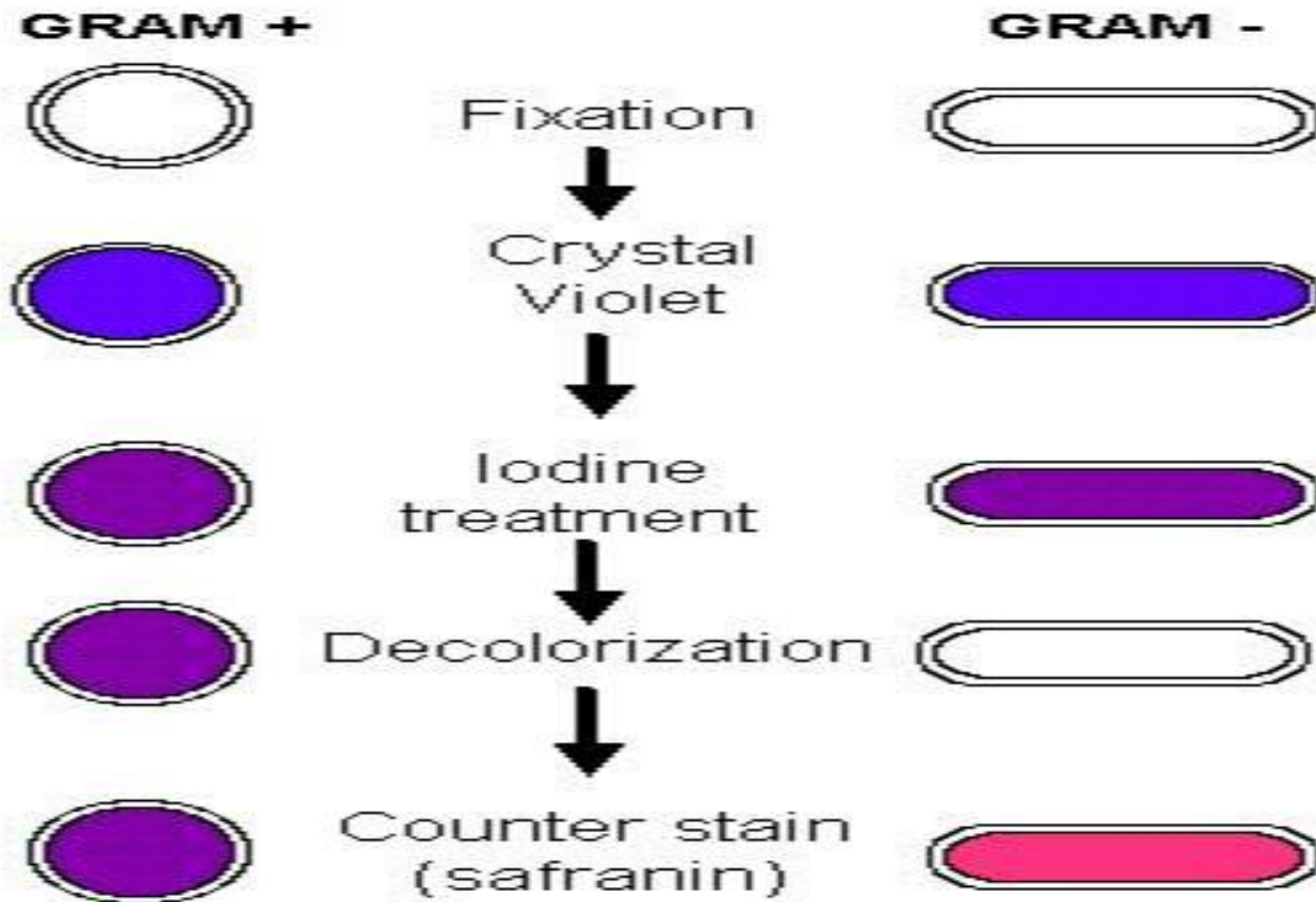


Pass slide through flame to fix

Con't..

- Flood the fixed smear with crystal violet solution and allow to remain for 1 minute
- Wash off the crystal violet with tap water.
- Flood the slide with iodine solution for one minute.
- Wash off the iodine solution with tap water.
- Flood the slide with decolorizer (alcohol) for one to 5
- Wash off the decolorizer with tap water
- Flood the slide with safranin for 30 seconds.
- Rinse off the safranin with tap water.
- Dry the slide on bibulous paper and place in an upright position
- Examine the slide for bacterial organisms under a 100X objective microscope. G+= violet / blue, G- = pink /red

Cond'



- **Acid-Fast stain:**(also called the Ziehl-Neelsen stain)
it is used to stain , those in the genus mycobacterium do not bind simple stains readily and must be stained by a harsher treatment. This is due to the high lipid content of acid-fast cell walls; in particular high content of mycolic acid

Staining specific structures

Negative staining: this technique reveals the presence of the diffused capsules surrounding many bacteria.

Spore staining

By Schaeffer Fulton procedure

- ✓ Endospores are first stained by heating bacteria with malachite green
- ✓ After malachite green treatment, the rest of the cell is washed free of dye with water
- ✓ Then counter stained with safranin.
- ✓ Result: will be a green endospore resting in a pink to red cell.

•Rickettsia

- ✓ Small in size,
- ✓ gram-negative
- ✓ obligate intracellular bacteria that replicate only in living cells.
- ✓ Non-motile
- ✓ non capsulated
- ✓ They are host specific and have tropism for particular cell types.
- ✓ Do not survive in extracellular environment except *Coxiella burnetii*
- ✓ Cause systemic diseases, mainly arthropod-borne, in humans and animals
- ✓

Cond'

- Many *Rickettsia* species are primarily pathogens of humans.
- Produce latent infections.
- In arthropods, rickettsia replicate in the epithelial cells of the gut before spreading to other organs including the salivary gland and ovaries.
- Aerosol transmission of *C. burnetii* commonly occurs in domestic animals and humans

Culturing

- ✓ unable to grow in cell free media
- ✓ well cultivated in the yolk sack of developed chick embryos, on mouse fibrioblast, HeLa and other continous cell lines
- ✓ Growth takes place in the infected cells.
- ✓ The optimum temperature for growth is 32-35 °C
- ✓ Laboratory animals (guinea pigs and mice) are useful for isolation of rickettsiae from patients

Diseases Caused by Rickettsia species

species	hosts	Diseases
<i>Rickettsia rickettsii</i>	humans dogs	Rocky Mountain spotted fever
<i>R. prowazekii</i> ,	humans	typhus
<i>R. typhi</i>	humans	typhus
<i>Ehrlichia ruminantum</i>	young animals (calves and lambs)	heart water or cowdriosis in
<i>Coxiella burnetii</i>	in farmers abattoir workers veterinarians	Q-fever. (It is influenza like occupational disease)
<i>Ehrlichia canis</i>	canine	canine monocytic ehrlichiosis

Mycoplasma

- ✓ The mycoplasmas, The smallest prokaryotic cells capable of self-replication
- ✓ Pleomorphic: exist
Spherical= 0.3 to 0.9 μm in diameter to filamentous =up to 1 μm long).
- ✓ do not possess rigid cell walls
- ✓ but have flexible triple-layered outer membranes.
- ✓ Their flexibility allows them to pass through bacterial membrane filters of pore size 0.22 to 0.45 μm .

Cond'

- ✓ resistant to antibiotics such as penicillin.
- ✓ facultative anaerobic and require exogenous sterols supplied by animal serum added to the growth medium.
- ✓ grow slowly, with a generation time of 1 to 6 hours, and most form small colonies that have a fried-egg appearance.
- ✓ Do not stain by gram method.
- ✓ Stained by Giemsa and other Romanowsky stain methods
- ✓ They are host specific

Diseases and principal hosts of the mycoplasmas

species	hosts	Diseases
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> (small colony type)	cattle	contagious bovine pleuropneumonia (CBPP)
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> (large-colony type)	cattle Sheep Goats	mastitis, pneumnpnia, or arthritis
<i>Mycoplasma mycoides</i> subsp <i>capri</i>	Goats	pleuropneumonia
Mycoplasma strain F-38	Goats	CCPP -Africa
<i>Mycoplasma gallisepticum</i>	chickens, turkeys	chronic respiratory disease
<i>M.agalactae</i> <i>M.conjunctivae</i>	Sheep Goats	Agalactia keratoconjunctivitis

- **Culturing**

- ✓ Mycoplasmas fastidious require specific growth factors, an isotonic medium.
- ✓ require a sterol and this is usually supplied by 20% horse serum.
- ✓ Other growth factors are catered(provideed) for by the addition of yeast extract.
- ✓ .The inoculated agar plates are incubated in a humid atmosphere at 37°c. aerobically under 5% CO₂ and 95% N₂

Mycology

By Betelihem tegege (DVM, MVSc in veterinary microbiology)

Introduction

- **Mycology** is the study of fungi and it deals with
 - ✓ Morphology, Reproduction, Physiology
 - ✓ Classification of fungi, and the diseases caused by them.
- Fungi are eukaryotic and their cell contains a true membrane-bound nucleus and other organelles

- Fungi may be either unicellular or multicellular. Multicellular fungi produce filamentous microscopic structures called moulds; yeasts which are unicellular, have a spherical or ovoid shape and multiply by budding.
- Dimorphic fungi occur in both mould and yeast forms. Environmental factors usually determine the form in which a dimorphic fungus occurs. Fungi such as *Candida albicans*, which produce forms additional to the two major forms, are described as poly- morphic.

- A notable feature of fungi is their ability to secrete potent enzymes which can digest organic matter. When moisture is present and other environmental conditions are favorable, fungi can degrade a wide variety of organic substrates.

- A small number of yeasts and moulds are pathogenic for humans and animals. To be pathogenic, they must tolerate the temperature inside the host and possess enzymatic system that allows them to parasitize animal tissues.
- Some fungi invade tissues whereas others produce toxic.

Importance of fungi

- Fungi have been cultivated for centuries for food, to produce antibiotics and other drugs, to make bread rise, and to ferment beer and wine.
- Fungi play ecological diverse roles since they are decomposers(saprophytes) and mutualistic symbionts.
- Saprophytic fungi absorb nutrients from nonliving organisms particularly dead plants and recycle them.

- Many antibiotics, including penicillin, are derived from fungi (Penicillium).
- Can also be used as natural food supply for wild animals
- Used in bioremediation (reduces toxic concentration)
- Used in agriculture, horticulture and forestry (biofertilizers and biopesticides)

General characteristics of Fungi

- Fungi are non-photo-synthetic heterotrophs which produce exoenzymes and obtain nutrients by absorption. They can exist as: Saprophytes, Parasites or Commensals.
- Fungi are non-motile
- They grow aerobically, and many are strict aerobes.
- Growth rate of fungi is slower than that of bacteria

- They can reproduce both sexually or asexually.
- They possess rigid cell wall containing chitin, mannan glucan and other polysaccharides. Unlike bacteria, Fungal cell wall doesn't contain peptidoglycan.

- Fungi perform extra-cellular digestion and absorb their nutrients.
- Catabolic enzymes outside their bodies will break large organic molecules into smaller molecules. The smaller molecules are then absorbed through the cell membrane.

Growth conditions for fungi

Fungal group	Incubation conditions	
	Temperature (°C)	Time
Dermatophytes	25	2 to 4 weeks
<i>Aspergillus</i> species	37	1 to 4 days
Yeasts (pathogenic)	37	1 to 4 days
Dimorphic fungi		
mould phase	25	1 to 4 weeks
yeast phase	37	1 to 4 weeks
Zygomycetes	37	1 to 4 days

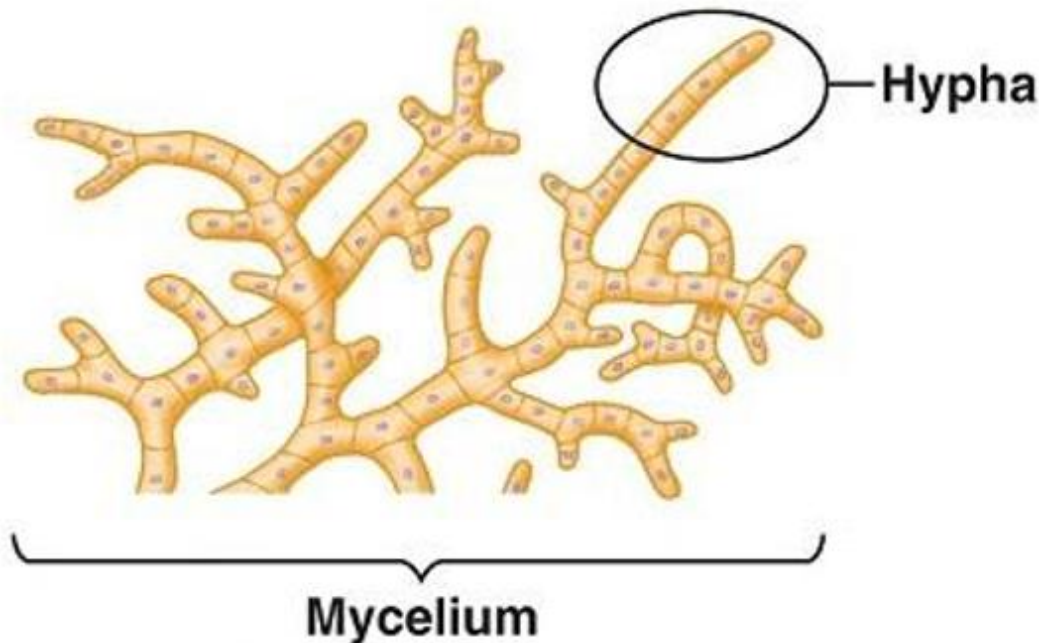
- Although there are many species in the kingdom Fungi, only few are known to be pathogenic for animals and man.
- The diseases caused by fungi are called mycoses. They are resistant to antimicrobial drugs which are effective against bacteria

Structure of Fungi:

- Hyphal cell walls, which impart rigidity and osmotic stability, are mainly composed of carbohydrate components including chitin macromolecules with cellulose cross-linkages.
- In yeasts, cell walls contain protein complexed with polysaccharides and, in some species, a range of lipid compounds.

- In the bi layered cell membrane, which lines the cell wall in the fungi, the predominant sterol is ergosterol in contrast to cholesterol, which predominates in the cell membranes of animals.
- Both moulds and yeasts have nuclei with well-defined nuclear membranes, mitochondria and networks of micro- tubules.

- Septa (cross-walls) are often present in hyphae (septate). They can also be aseptate (lacking cross walls). Extension of hyphae and their lateral branches results in the formation of a mycelium, an interlacing network of hyphae.



Reproduction:

- Fungi can reproduce asexually and/or sexually.
- Fungi most commonly reproduce by the formation of spores.
- A spore is: a reproductive cell that is capable of growing into a new organism by mitotic division alone. Reproduction by spore formation may be either sexual or asexual. In some species both types of spore formation occur.

- Moulds tend to form large colonies with growth and extension of hyphae at their peripheries. In some species, mature elements at the centre of colonies produce specialized aerial hyphae which support spore-bearing structures and facilitate dispersal of mature spores.

- In this asexual reproduction, two main types of spores, conidia and sporangiospores are recognized.
- Conidia are formed on conidiophores and sporangiospores are formed within a sporangium, a sac like structure borne on an aerial hypha termed a sporangiophore.

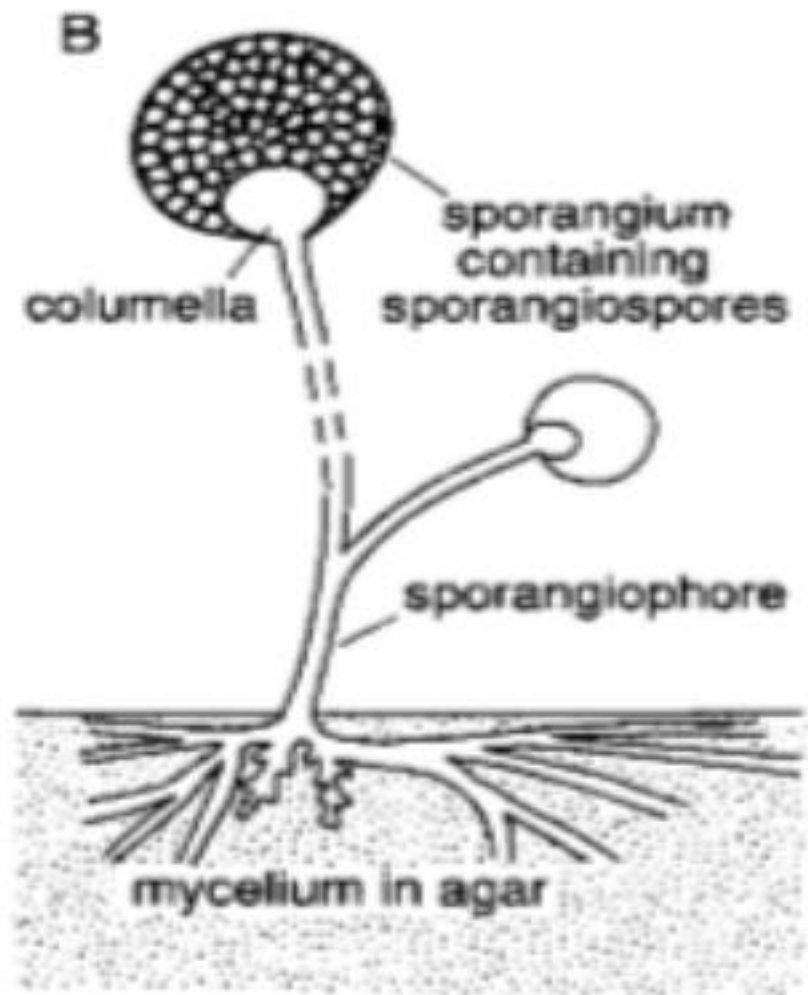
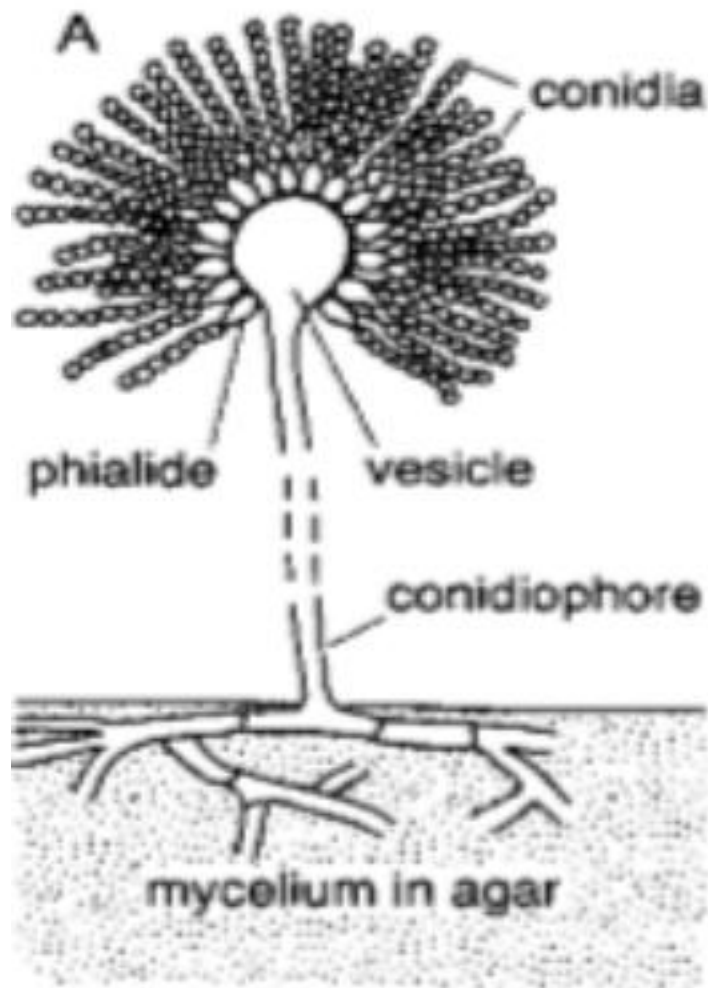
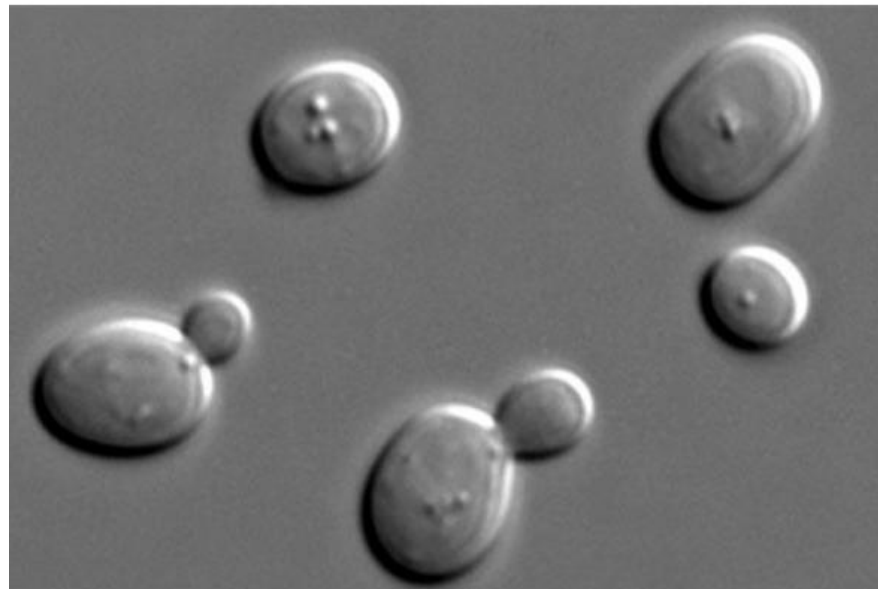


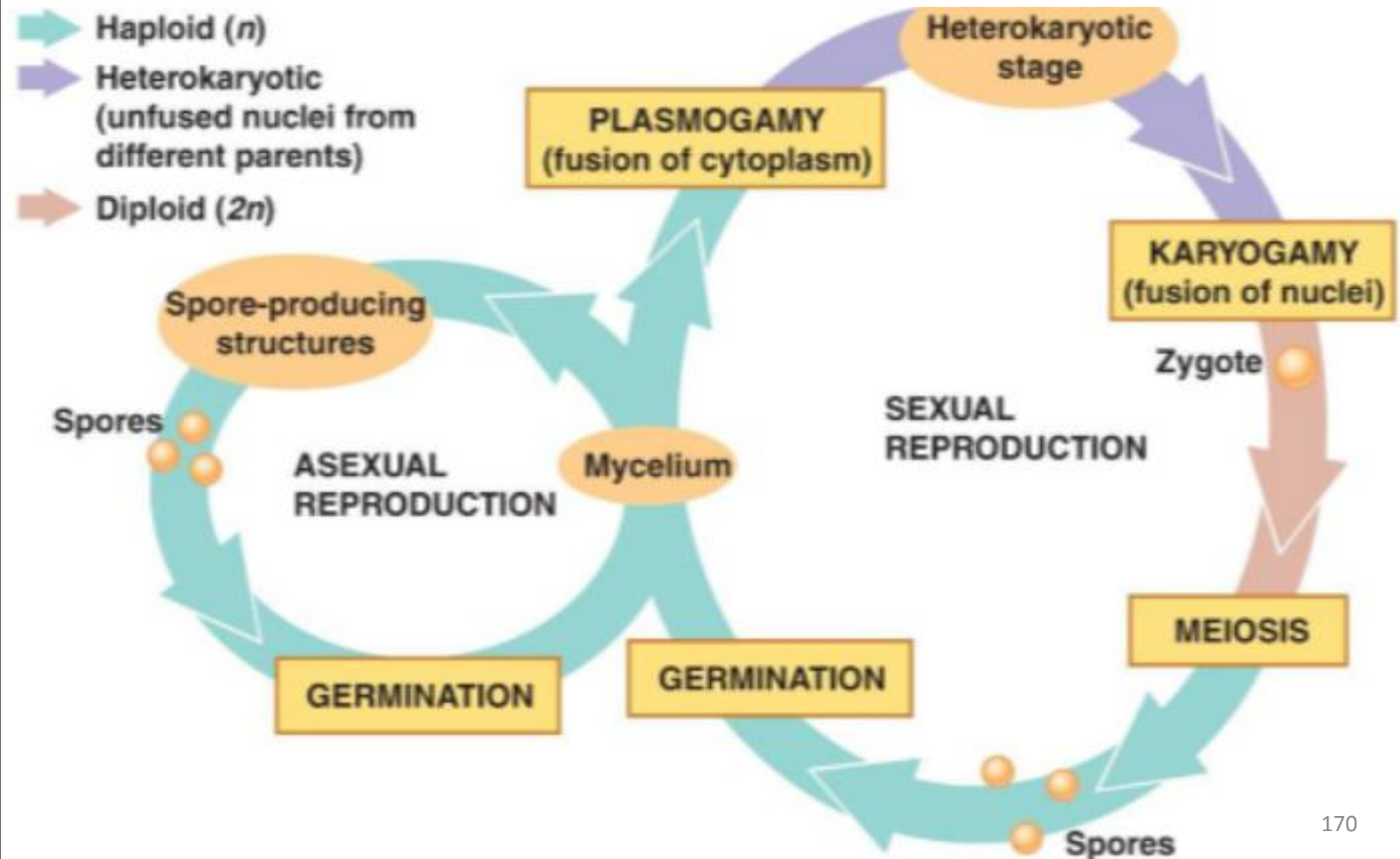
Figure 37.3 Fungal growth on agar illustrating vegetative mycelia and aerial hyphae with sporing heads. **A.** *Aspergillus* species. **B.** *Rhizopus* species.

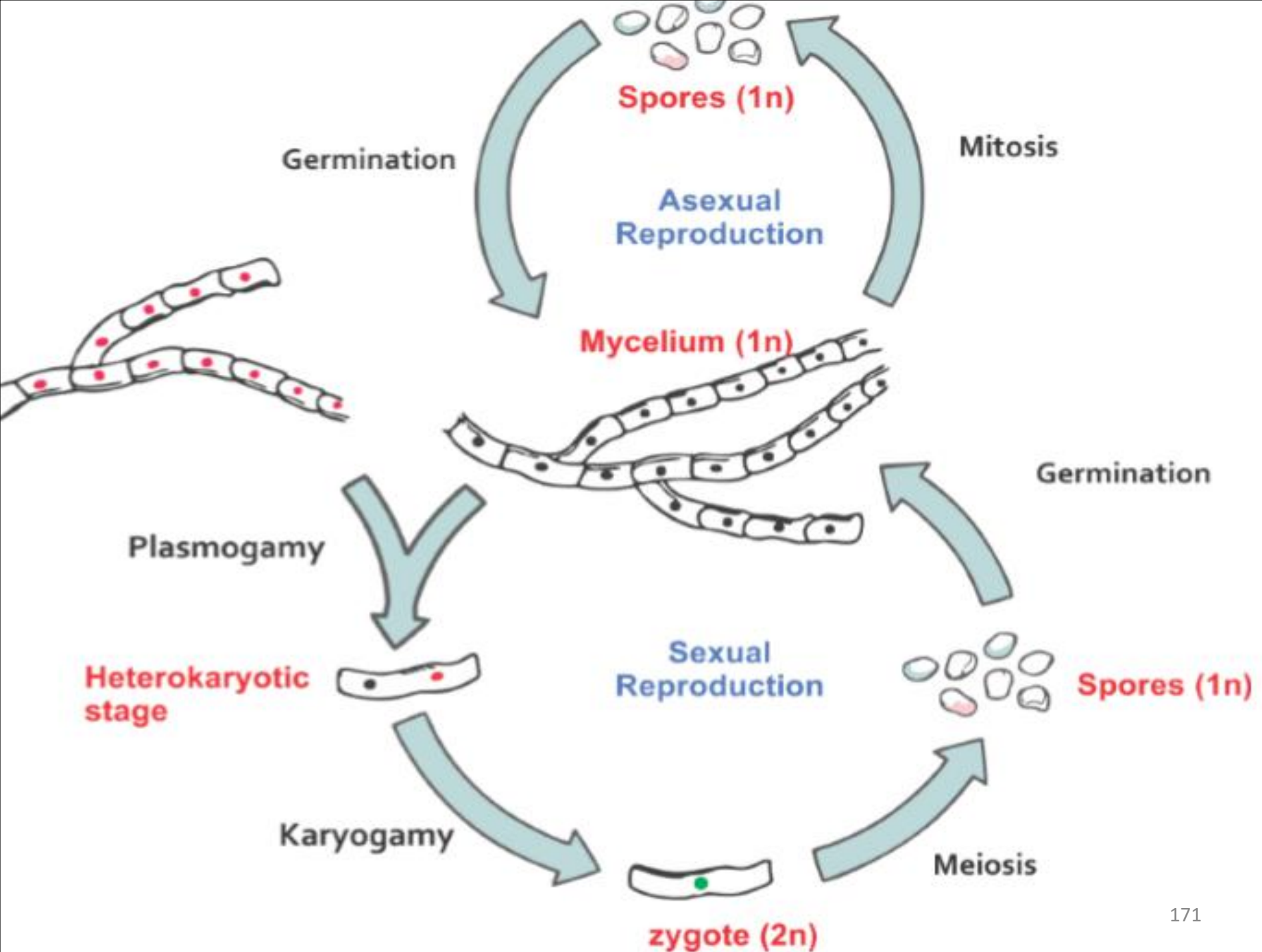
- Sporangiospores are formed only by fungi in the phylum Zygomycota.
- In Dermatophytes, multicellular structures called macro conidia and single celled micro conidia are produced in cultures from lateral hyphal branches, whereas arthro conidia are formed from the disintegration of hyphae within keratinized structures.

- In most yeasts, asexual division is by budding. Daughter cells separate from parent cells after the formation of a cross-wall at the point of budding. The colonies of yeast-like fungi are soft, smooth and round.



Sexual and asexual reproduction in fungi





Classification of Fungi

- Many systems of classification are in use. They are based on morphological, biochemical, and molecular traits of disease-causing fungi and are being continually refined as more and more phylogenetic criteria are recognized.
- Historically, the classification of fungi has largely been based on their morphology, rather than on the physiological and biochemical differences that are of such importance in bacterial classification

- The binomial names and taxonomic hierarchies provide useful information on disease diagnostics.
- They also provide information on biology and ecology of the disease-causing fungi, such as morphology, modes of dispersal/infection, survival of infectious ones, potential of evolution and toxicological/biochemical characteristics

A. Morphological Classification

On the basis of morphology, there are four groups of fungi:

1. Yeasts: Examples: *Saccharomyces cerevisiae* (non pathogenic yeast) and *Cryptococcus neoformans* (pathogenic yeast).

2. Yeast-like fungi: Yeast-like fungi grow partly as yeast and partly as elongated cells resembling hyphae. The latter form a pseudo mycelium. Example: *Candida albicans* is a pathogenic yeast-like fungus.

3. Molds or filamentous fungi: Examples: Dermatophytes, Aspegillus, Penicillium, Mucor and Rhizopus.

4. Dimorphic fungi: These fungi have a yeast form in the host tissue and in vitro at 37°C on enriched media and hyphal (mycelial) form in vitro at 25°C.
Example: *Histoplasma capsulatum*

B. Systematic Classification

On the basis of formation of sexual spores, fungi have been divided into 4 classes

1. Zygomycetes: Zygomycetes are lower fungi which have non septate hyphae and form asexual spores called sporangiospores contained within swollen oospores. Examples: Rhizopus, Absidia, Mucor, Pilobolus. Other three classes Ascomycetes, Basidiomycetes and Deuteromycetes or Fungi Imperfecti possess septate hyphae.

2. Ascomycetes: The Ascomycetes form sexual spores (ascospores) within a sac or ascus. Ascomycetes include both yeasts and filamentous fungi.

3. Basidiomycetes: The Basidiomycetes form sexual spores (basidiospores) on a basidium or base. Examples: Mushrooms, *Filobasidiella neoformans* (anamorph), *Cryptococcus neoformans*.

4. Deuteromycetes (Fungi Imperfecti): Fungi that lack a known sexual state are placed in the class Deuteromycetes (Fungi Imperfecti). The members of this class may have lost the ability to reproduce sexually or their sexual states may have not been discovered. Most fungi of medical importance belong to this class.

Examples: *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Candida albicans*.

C. Other systems of classification

- Main taxa of one of the current systems of classification for veterinary and medical pathogenic fungi are also in use.

Kingdom: Fungi

- Phylum: Chytridiomycota
- Phylum: Zygomycota
- Phylum: Ascomycota
- Phylum: Basidiomycota

- Kingdom Fungi are usually organized in a hierarchical manner, each rank being named with, and recognizable by, a particular ending: phylum, -mycota; subphylum, -mycotina; class, -mycetes; order, -ales; and family, -aceae. Each family is composed of a number of genera, and these are divided into species.

Fungal diseases (mycoses)

- Fungi are relatively uncommon causes of disease in healthy and immunocompetent animals and humans. However, an increasing number of recalcitrant fungal diseases in animals have occurred over the last two decades, originating from opportunistic and pathogenic fungi

- Opportunistic fungi have a preferred habitat independent from the living host and cause infection after accidentally penetration of intact skin barriers, or when immunologic defects or other debilitating conditions exist in the host.
- In contrast, pathogens are defined as having advantage of the vertebrate host; in obligatory pathogens the host is indispensable to complete their life cycle and for nutrient acquisition, growth and reproduction.

- Fungal zoonoses also exist which are infections that can be naturally transmitted between vertebrate animals and humans.

Classification of mycoses

- Groups of fungal diseases according to primary sites of infections: A. Superficial mycoses B. Cutaneous mycoses C. Subcutaneous mycoses D. Systemic mycoses E. Opportunistic mycoses.

A. Superficial Mycoses: These infections are limited to the outermost layers of the skin and hair. Example: Infection of skin caused by *Malassezia furfur* (pityriasis versicolor)

B. Cutaneous Mycoses: Infections that extend deeper into the epidermis as well as invasive hair and nail diseases.

Examples: a. Infection of skin, hair and nail caused by dermatophytes. b. Infection of skin, nail and mucous membrane caused by *C. albicans* and other *Candida* species.

C. Subcutaneous Mycoses: These infections involve the dermis, subcutaneous tissues, muscle, and fascia. Examples: Chromomycosis, Sporotrichosis and Rhinosporidiosis.

Dermatophytosis (ring worm) in cattle



D. Systemic Mycoses: Infections that originate primarily in the lung but that may spread to many organ systems. Examples: Systemic mycoses include blastomycosis, histoplasmosis, coccidioidomycosis and paracoccidioidomycosis.

E. Opportunistic Mycoses: Opportunistic infection occur in patients with debilitating diseases or in whom the physiological state has been upset by immunosuppressive drugs, steroids, X-rays or broad spectrum antibiotics.

- Opportunistic infections are caused mainly by fungi that are normally avirulent. Examples: Aspergillosis, Penicilliosis, Zygomycosis or Mucormycosis, Candidiasis and Cryptococcosis.

Mycotoxins and mycotoxicoses

- Mycotoxines are defined as chemicals of fungal origin being toxic for (warm-blooded) vertebrates
- Mycotoxins are secondary metabolites produced during consecutive enzyme reactions via several biochemically simple intermediary products from the primary metabolism of acetates, mevalonates, malonite, and some amino acids.
- The contamination of foods and animal feeds with mycotoxins is a worldwide problem,

- Formation of mycotoxins by many food spoilage fungi is one of the most significant risk factors to mammalian health.
- A single species of fungi may produce one or several mycotoxins and individual mycotoxins may be produced by different fungal species.

- Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids are main mycotoxins of public health and agro-economic importance.
- Mycotoxins cause intoxications in both animals and humans, resulting in severe diseases called mycotoxicoses.

- In poultry farms, contaminated feeds with aflatoxins to broilers causes negative metabolic responses and enzyme activity resulting reduced body weight gain, and tissue necrosis.
- In dogs, ingestion of a variety of moldy foods, including grains, walnuts, almonds, and peanuts, as well as non specific garbage, has been associated with tremorgenic mycotoxicosis.

General virology for animal science students

By Dr. Betelihem Tegegne (DVM, MVSc in veterinary microbiology)

Virology

Introduction

- Virology is a science that studies the characteristics of viruses including their morphology, physiology multiplication, nomenclature and classification of viruses, diseases caused by them and the measures fighting against them.

Con't..

Virus: (Latin, poison) is a non cellular particle made up of genetic material (DNA or RNA) and protein coat that can invade in living cells.

Characteristics

- ✓ Infectious agent, extremely small: 18 to 300 nm
- ✓ Obligate intracellular parasites
- ✓ Highly organized structure
- ✓ Inert or dormant outside their host cells
- ✓ Contain a single type of Nucleic Acid(genome): DNA or RNA
- ✓ Do not possess functional organelles
- ✓ Do not replicate on inert media since viable host cells are required for replication

Viruses have both living and non living characteristics

1. Living characteristics

- ✓ They reproduce (only in living host cells)
- ✓ They can mutate

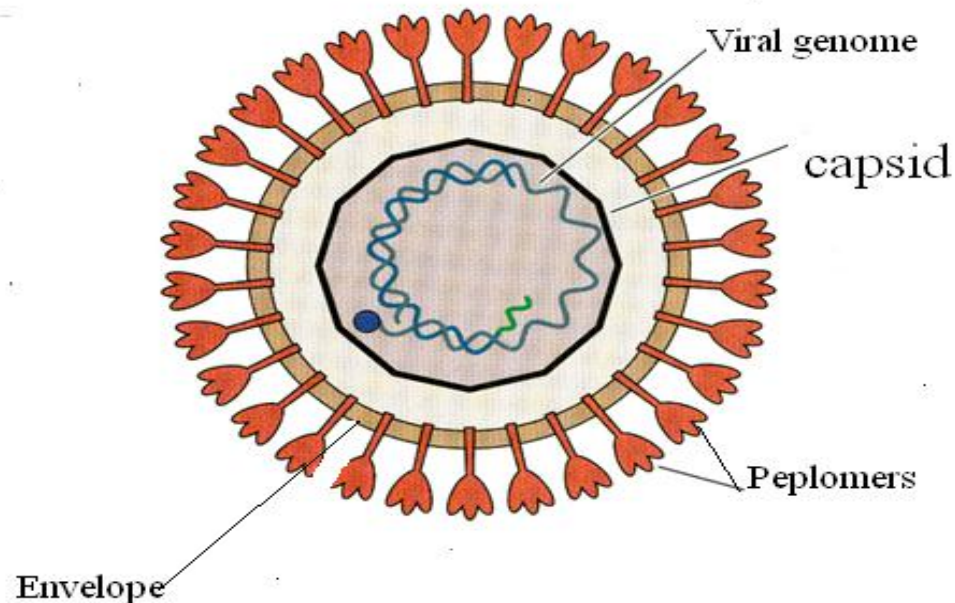
2. Non –living characteristics

- ✓ Acellular, that is they contain no cytoplasm or cellular organelles
- ✓ They carry out no metabolism on their own and must replicate using the host cell's metabolic machinery

Viral structure (Morphology)

- Structurally viruses are much simple than bacteria.

Structure of viral particle



A. Viral genome: A virus particle, also known as a virion, is essentially a nucleic acid (DNA or RNA) enclosed in a protein shell or coat. It can be single-stranded or double-stranded DNA or RNA ,but never both.

B. Capsid: The protein coat that envelopes or surrounds the viral nucleic acid (genetic material). Each capsid is composed of repeating protein subunits - capsomers or protomers.

Symmetry of capsid: differs with different viruses

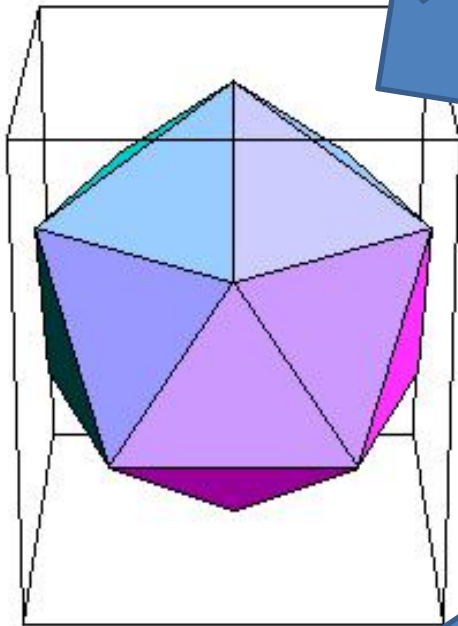
Con't..

The capsid symmetry recognized in viruses are:

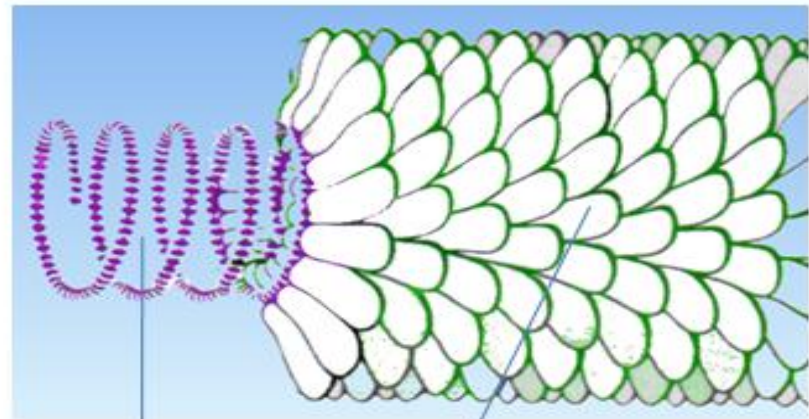
- ✓ Icosahedral
- ✓ Helical and
- ✓ **Complex:** Viruses that show neither icosahedral nor helical symmetry due to their complex structure

Icosahedral symmetry: Viruses with Icosahedral symmetry have 20 equilateral triangular faces, 12 corners or apices and 30 edges

Icosahedral



Helical structure of virus



coiled RNA

protein subunits

Helical symmetry

- The nucleic acid and the capsomers are wound together in the form of helix or spiral

C. Envelope: a lipid bi-layer that usually covers the capsid of some types of viruses

Viral taxonomy and nomenclature

Virus classification involves naming and placing viruses into a taxonomic system.

- ✓ Virus classification is based mainly on phenotypic characteristics.

Some properties of viruses used in taxonomy

1. Virion properties:

- ✓ Size, presence or absence of envelope and peplomers,
Capsid symmetry and structure

2. Properties of Genome:

- ✓ Type of nucleic acid (RNA or DNA); Size and strand
(double or single stranded)

3. Properties of Proteins:

- ✓ Number, size, enzymatic activity, Modification

4. Replication strategy

- Viral Strategy of replication, Transcriptional characteristics, translational characteristics can also be used for classification of viruses

- The international committee on taxonomy of viruses (ICTV) sets the universal system of viral taxonomy as follows

□ The name of each level ends with specific suffix.

- Orders-----virales
- Families----- viridae
- Subfamily ----- virinae
- Genus -----virus
- Species-----virus

Example: 1

Order—*Mononegaviriales*

Family---*Rhabdoviridae*

Genus---*Lyssa virus*

Species-----rabies virus

Example 2:

Family—Poxviridae,

Subfamily-- *Chordopoxvirinae*

genus---*Suipoxvirus*,

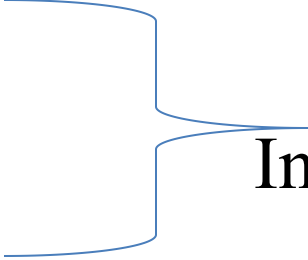
species----swinepox virus

DNA & RNA virus families

DNA virus families	RNA virus families
1. Adenoviridae	1. Reoviridae
2. Papillomaviridae	2. Picornaviridae
3. Herpesviridae	3. Caliciviridae
4. Parvoviridae	4. Togaviridae
5. Poxviridae	5. Arenaviridae
6. Iridoviridae	6. Flaviviridae
	7. Orthomyxoviridae
	8. Paramyxoviridae
	9. Bunyaviridae
	10. Rhabdoviridae
	11. Filoviridae
	12. Coronaviridae
	13. Birnaviridae

Viral Replication

- Viruses need a living host cell to multiply and each infected cell may produce as many as 100,000 virus.
- There are five major steps in the replication cycle of all viruses

- ✓ Attachment
 - ✓ Penetration (Entry)
 - ✓ Uncoating
 - ✓ Replication (Multiplication) or synthesis
 - ✓ Assembly
 - ✓ Release
- 
- Initiation phase

Attachment:

- Do not require energy from host cells but it is electrostatic
- Viral proteins on the capsid or phospholipid envelope interact with specific receptors on the host cellular surface.

Penetration:

- Refers to the entry of viral particles into the cytoplasm of host cell

- ✓ Energy dependent
- Viruses may penetrate to host cells by:
 - Endocytosis or
 - Fusion

Uncoating:

- ✓ Refers the physical separation of the viral capsid from the viral genome (removal or degradation of capsid)
- ✓ Obligatory step in viral replication
- ✓ The viral genome is transported to the site where transcription/ replication can begin
- ✓ Favored low pH

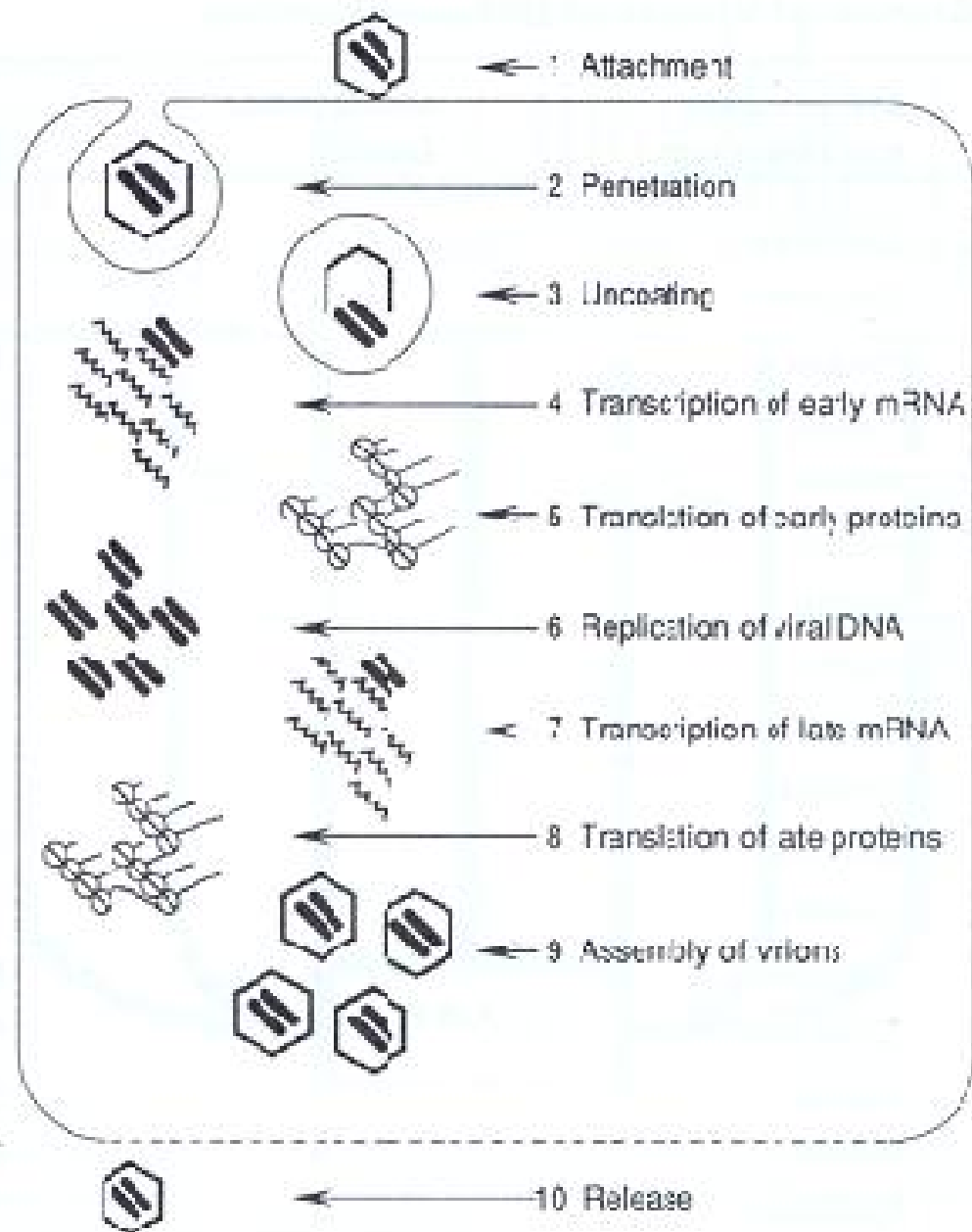
Synthesis or replication

This phase includes synthesis of

- ✓ Viral nucleic acid (DNA or RNA)
- ✓ Capsid protein
- ✓ Enzymes necessary in the various stages of viral synthesis, assembly and release
- ✓ Regulatory proteins which serve to shut down the normal cellular metabolism

Con't..

- Most DNA viruses except poxviruses replicate mainly in the nucleus and use the cell's DNA-dependent RNA polymerase and other enzymes to make mRNA
- Most RNA viruses except Orthomyxoviruses and retroviruses replicate in the cytoplasm of infected cells. RNA viruses encode the necessary enzymes for transcription and replication because cell has no means of replicating RNA



General feature of the viral replication cycle (DNA), using adenovirus as a model

Cell membrane

Receptors

Spikes

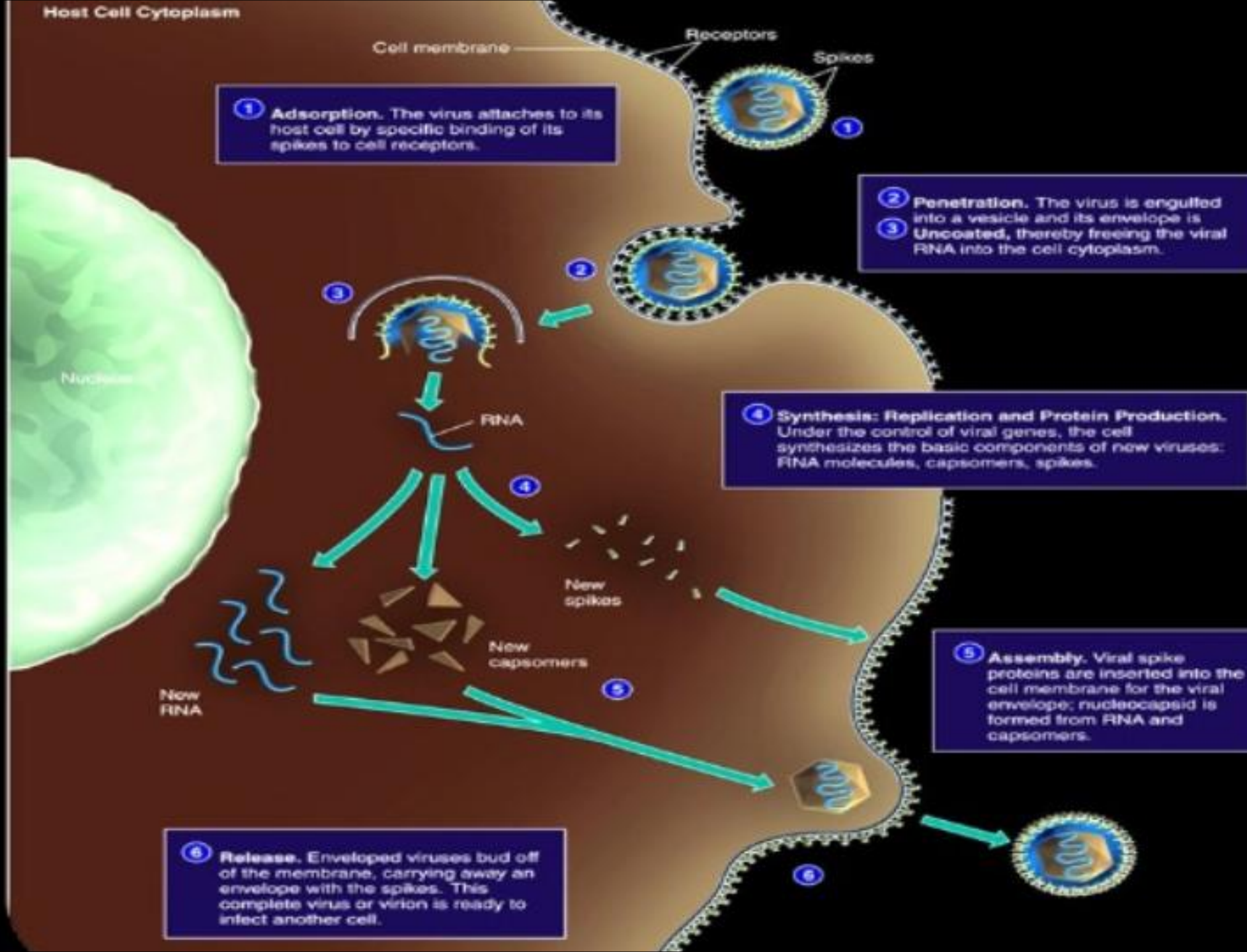
1 Adsorption. The virus attaches to its host cell by specific binding of its spikes to cell receptors.

2 Penetration. The virus is engulfed into a vesicle and its envelope is **Uncoated**, thereby freeing the viral RNA into the cell cytoplasm.

4 Synthesis: Replication and Protein Production. Under the control of viral genes, the cell synthesizes the basic components of new viruses: RNA molecules, capsomers, spikes.

5 Assembly. Viral spike proteins are inserted into the cell membrane for the viral envelope; nucleocapsid is formed from RNA and capsomers.

6 Release. Enveloped viruses bud off of the membrane, carrying away an envelope with the spikes. This complete virus or virion is ready to infect another cell.



Cultivation of viruses

- Viruses can grow and replicate only in living cells.
So viable or living cells are required for their cultivation.
- ✓ Cell culture and tissue culture
- ✓ Embryonated chicken eggs
- ✓ Experimental animals are employed for the isolation and propagation of particular viruses

- Cultivation (propagation) of viruses is required for
 - ✓ Isolation and identification of viruses involved in disease
 - ✓ The titration of viruses for vaccine production
 - ✓ The provision of stocks for research purpose

Cell Culture

- Cells are the most widely used and most powerful hosts for cultivation of viruses.
- Three basic types
 - ✓ Primary cell culture (like bovine kidney and lamb testis)
 - ✓ Cell lines (continuous cell culture) like vero cell line
 - ✓ Cell strains (semi continuous cell culture)

Con't..

- Viral growth in cell culture is recognized in change of shape, cell detachment, fusion leading to syncytia formation, presence of inclusion bodies and cell death

Embryonated chicken eggs

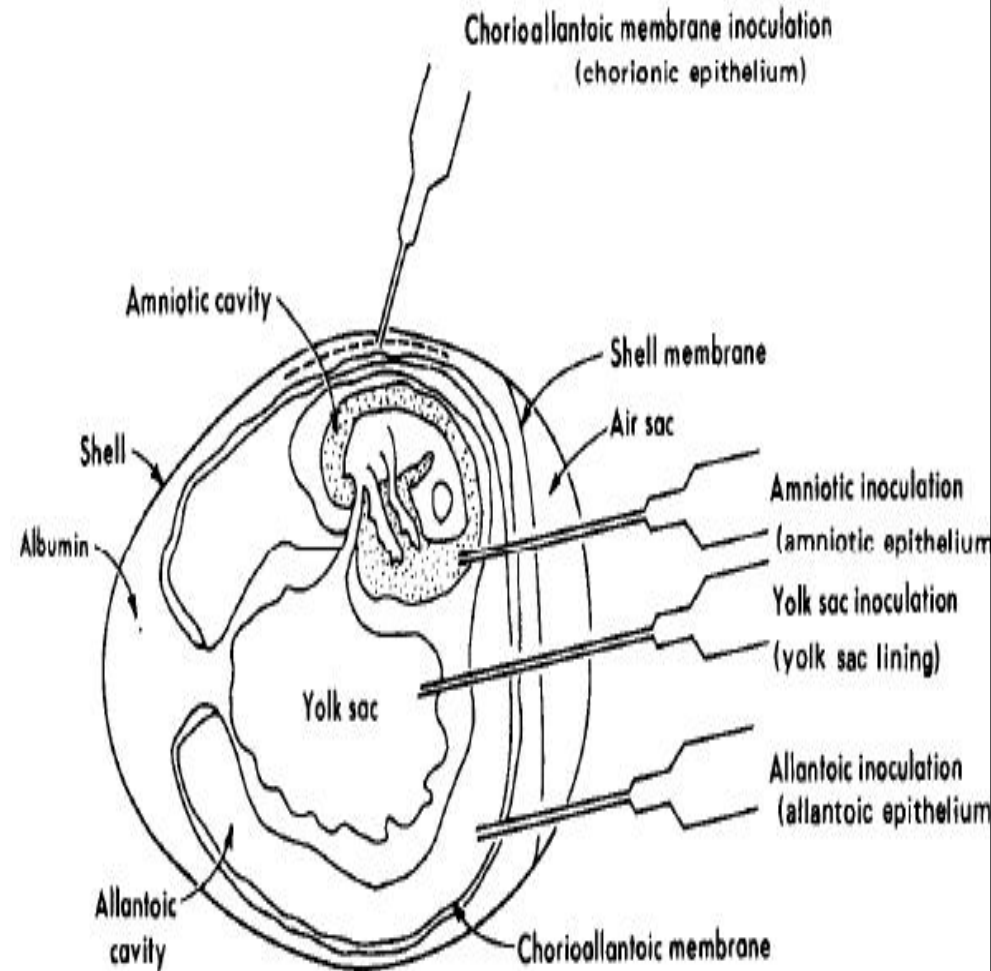
- ✓ It is used extensively for virus isolation
- ✓ Fertilized eggs at 10- 14 days are employed and incubated for 2-9 days after inoculation

Embryonated chicken eggs
may be inoculated into

- ✓ Allantoic cavity
- ✓ Amniotic cavity
- ✓ Chorioallantoic membrane
- ✓ Yolk sac and
- ✓ Intravascular in well developed chick embryos

Result

- ✓ Dwarfing of the embryo
- ✓ Formation of pocks on CAM
- ✓ Death



Laboratory (Experimental) animals

- Prior to the advent of cell culture and embryonated chicken eggs, animals' viruses could be propagated on whole animals.
- Whole animals could include the natural host or the laboratory animals such as, Rabbits, Mice, Rats, Hamsters and Guinea pigs

Pathogenesis of viral infections

- **Viral pathogenesis:** includes processes by which viruses produce disease in the host
- **Virulence:** is the ability of a virus to produce disease in a host
- Virulence of a virus depends on
 - ✓ The dose and route of entry of the virus
 - ✓ The immune status, species, breed, age and physiological status of the host

Viral pathogenesis steps

Infection of susceptible host with viral pathogen



Replication at site of tissue invasion



Spread to regional lymph nodes and replication



Entry to blood stream



Primary viremia



Replication in bone marrow, spleen and other organs



Secondary viremia



Lesions in target organs and characteristic clinical sign

Some examples of viral diseases common in animals

In cattle sheep and goats

- Sheep pox, goat pox and lumpy skin disease, orf (Caused by viruses in the poxviridae family), peste des petits ruminants (PPR), foot and mouth disease (FMD), rabies, blue tongue, rift valley fever...etc..

Poultry

- Avian influenza, Newcastle disease, marek's disease...

Dog and cats

- Rabies, canine distemper...

Equine

- African horse sickness, equine infectious anemia...

Laboratory diagnosis of viruses

- Tests that are used to identify infectious virus, viral antigen or viral nucleic acids include
- EM ----Electron microscopy
- PCR--- Polymerase chain reaction
- VN---- Virus neutralization
- HA----Hemagglutination
- CFT----Compliment fixation test

- AGID----Agar gel immuno diffusion
- ELISA----Enzyme linked immuno sorbent assay
- RIA-----Radioimmunoassay
- HAI-----Hemagglutination inhibition
- IFAT-----Indirect fluorescence antibody test

Control of viral infections

- ✓ There are usually no curative treatments for viral diseases but to control and prevent dissemination we can use
 - ✓ Vaccination
 - ✓ Preventing the unrestrained movement of animals and their products out of endemic areas
 - ✓ Replacement animals should be purchased from disease free areas or farms

- ✓ Quarantine of newly introduce animals to the farm
- ✓ Isolation of sick animals from the herd
- ✓ Proper disposal of cadaver (if any) and dirt (feces, urine, hair, feathers and so on that may be contaminated with viruses) timely from the farms
e.t.c
- ✓ Elimination of arthropod vectors

Veterinary Immunology

By Dr. Betelihem Tegegne

The basics of Immunology

Brief history of immunology

- The discipline of immunology grew out of the observation that individuals who had recovered from certain infectious diseases were thereafter protected from the disease.
- The Latin term *immunis*, meaning “exempt” is the source of the English word *immunity*, meaning the state of protection from infectious disease. The scientific study of the immunity/ body defense is immunology.

Con't..

- When infections such as smallpox or plague spread through early human societies, many people died, but some individuals recovered. It was rarely noticed that these recovered individuals remained healthy during subsequent outbreaks—a sign that they had developed immunity.

Con't..

- The earliest recorded is about Thucydides, the great historian of the Peloponnesian War. In describing a plague in Athens, he wrote in 430 BC that only those who had recovered from the plague could nurse the sick because they would not contract the disease a second time.

- By the 12th century, the Chinese had observed that persons who recovered from smallpox were resistant to further attacks of this disease. So, they deliberately infected infants with smallpox by inserting scabs from infected individuals into small cuts in their skin. Those infants who survived the resulting disease were protected from smallpox in later life.

Con't..

- In 1718, Lady Mary Wortley Montagu observed the positive effects of variolation (inserting lesions to the skin of healthy individuals) on the native population and had the technique performed on her own children. The method was significantly improved by the English physician Edward Jenner, in 1798.

Con't..

- In 1798, Edward Jenner demonstrated that material from cowpox lesions could be substituted for smallpox in variolation. Since cowpox does not cause severe disease in humans, its use reduced the risks caused by variolation to insignificant levels.
- The effectiveness of this procedure, called vaccination (vacca is Latin for “cow”) was such that it was eventually used in the 1970s to eradicate smallpox from the world.

- In the year 1879, Louis Pasteur in France investigated fowl cholera, a disease caused by the bacterium *Pasteurella multocida*.

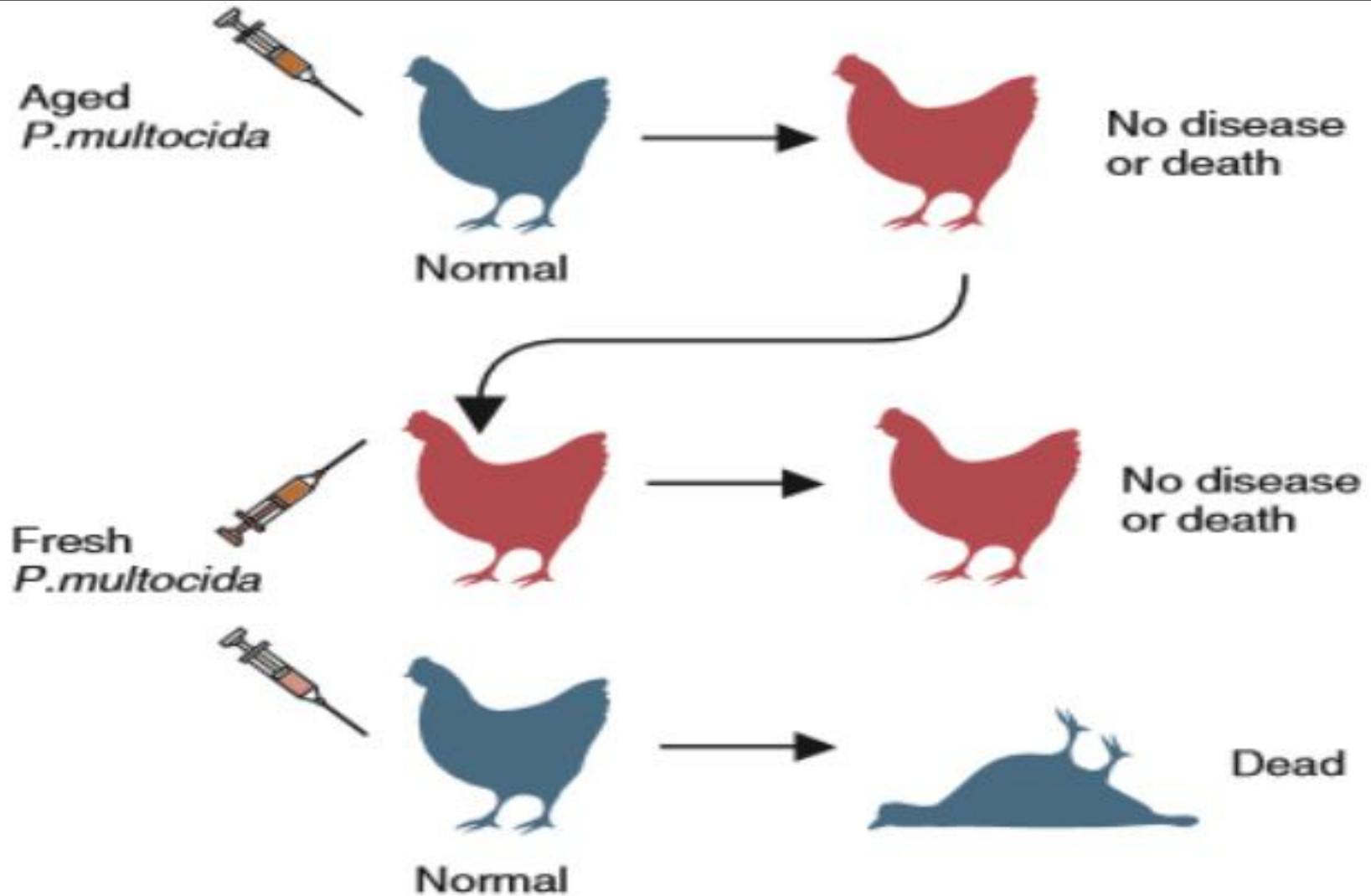


FIGURE 1-2 Pasteur's fowl cholera experiment. Birds inoculated with an aged culture of *Pasteurella multocida* did not die. However, when subsequently inoculated with a fresh culture of virulent *P. multocida*, the birds were found to be protected. It was this experiment that launched the science of immunology.

- By 1900 many vaccines had been developed, and the development of immunity to infectious diseases of animals was a well-recognized phenomenon. Since then, immunologists have determined the molecular and cellular basis of this antimicrobial immunity.
- This understanding has brought the ability to use immune mechanisms to enhance resistance to infectious diseases. The role of the immune system in many different disease processes has been clarified.

Immunity (immune response)

- The defenses of the body, collectively called the immune system, consist of complex, interacting networks of biochemical and cellular reactions.
- Immunity refers to the state of protection from infectious diseases. Understanding immunity requires an understanding of dynamic immunological networks. These networks possess redundancies and multiple simultaneous mechanisms working together to ensure microbial destruction.

- Multiple mechanisms are needed to ensure freedom from invasion. These include physical barriers that exclude invaders, innate immunity that provides rapid initial protection, and adaptive immunity that provides prolonged effective immunity.

Physical Barriers

- The body employs multiple, overlapping layers of defense.
- The first and most obvious of these defenses are the physical barriers to invasion. Thus intact skin provides an effective barrier to microbial invasion. If skin is damaged, microbes may invade; however, wound healing ensures that this is repaired very rapidly.

- On other body surfaces, such as in the respiratory and gastrointestinal tracts, simple physical defenses include the “self-cleaning” processes: coughing, sneezing, and mucus flow in the respiratory tract; vomiting and diarrhea in the gastrointestinal tract; and urine flow in the urinary system.

- The presence of a huge population of commensal bacteria on the skin and in the intestine also excludes many potential invaders. Well-adapted commensal organisms adapted to living on body surfaces can easily outcompete poorly adapted pathogenic organisms.

Innate Immunity

- Physical barriers cannot be totally effective in themselves. Invading microorganisms will eventually overcome physical obstacles. So, most microbial attempts at invasion should be blocked before they can result in disease. This is the task of the innate immune system. Many different innate defense mechanisms have evolved over time.

- The mammalian innate immune system is a collection of distinct subsystems that work through diverse mechanisms. Innate immunity is activated immediately when a pathogen penetrates the epithelial barriers, it ideally lasts for just a few hours, and is directed toward the rapid elimination of the pathogen.

- Innate immune mechanisms in general rely on the fact that microbes such as bacteria and viruses differ structurally and chemically from normal animal tissues.
- Animals make molecules that can kill invaders directly or promote their destruction by defensive cells.

Con't..

- Phagocytic cells, such as macrophages and neutrophils and a variety of antimicrobial compounds synthesized are parts of innate immunity
- Other innate subsystems include the complement system, a set of complex enzyme pathways that are lethal to invaders.
- Some of the cells involved in inflammation may also help repair damaged tissues once the invading microbes have been destroyed.

- Innate immunity components are not specific to a particular pathogen and have no any memory regarding a specific pathogen.

Adaptive Immunity

- Inflammation and the other subsystems of the innate immune system are critical to the defense of the body. Animals that cannot develop effective innate responses may die from overwhelming infections.

- These innate mechanisms cannot offer the ultimate solution to the defense of the body. What is really needed is a defense system that can recognize and destroy invaders and then learn from the process, so that if they invade again, they will be destroyed even more effectively.

- In this system, the more often an individual encounters an invader, the more effective will be its defenses against that organism. This type of response is the function of the adaptive immune system, so called since it adapts itself to the requirements of the animal (also called the acquired immune system)

Con't..

- The adaptive immune system takes several days or weeks to become effective. Although it develops slowly, when an animal eventually develops adaptive immunity to an invader, the chances of successful invasion by that organism decline and the animal is said to be immune.

- The adaptive immune system is a complex and sophisticated system that provides the ultimate defense of the body. The loss of adaptive immunity leads inevitably to uncontrolled infections and death.
- One form of adaptive immunity is mediated by antibodies. Antibodies are proteins that circulate in body fluids, especially in the bloodstream. They bind to bacteria and mark them for destruction.

- Another form of adaptive immunity is mainly directed against viruses. It is called cell-mediated immunity. This employs cells that destroy abnormal cells such as those infected by viruses.
- The adaptive immune system can remember prior exposure

□ **Table 1-1** | **Comparison of Innate and Adaptive Immunity**

	INNATE IMMUNITY ALWAYS "ON"	ADAPTIVE IMMUNITY TURNED ON BY ANTIGENS
Cells engaged	Macrophages, dendritic cells neutrophils, natural killer cells	T and B cells
Evolutionary history	Ancient	Recent
Onset	Rapid (minutes to hours)	Slow (days to weeks)
Specificity	Common microbial structures	Unique antigens
Potency	May be overwhelmed	Rarely overwhelmed
Memory	None	Significant memory
Effectiveness	Does not improve	Improves with exposure

Cells of immune response

- ✓ lymphocytes
- ✓ Antigen presenting cells

Lymphocytes are two types

- ✓ B- Lymphocytes(Bcells)
- ✓ T- Lymphocytes(T cells)
- ✓ B cells mature in bone marrow
- ✓ Membrane bound antibody molecule

- **T cells:**

- ✓ Arise in the bone marrow
- ✓ Migrate to the thymus gland to mature subpopulation of T cell
- ✓ T helper(TH) ,T killer(Tc) & T suppressor(Ts)
 - ✓ **antigenic presenting cells**
 - Activation of T helper cells

immunization

Immunization: A method of stimulating resistance in the animals body to specific diseases using mos – bacteria , viruses

Types of Immunization:

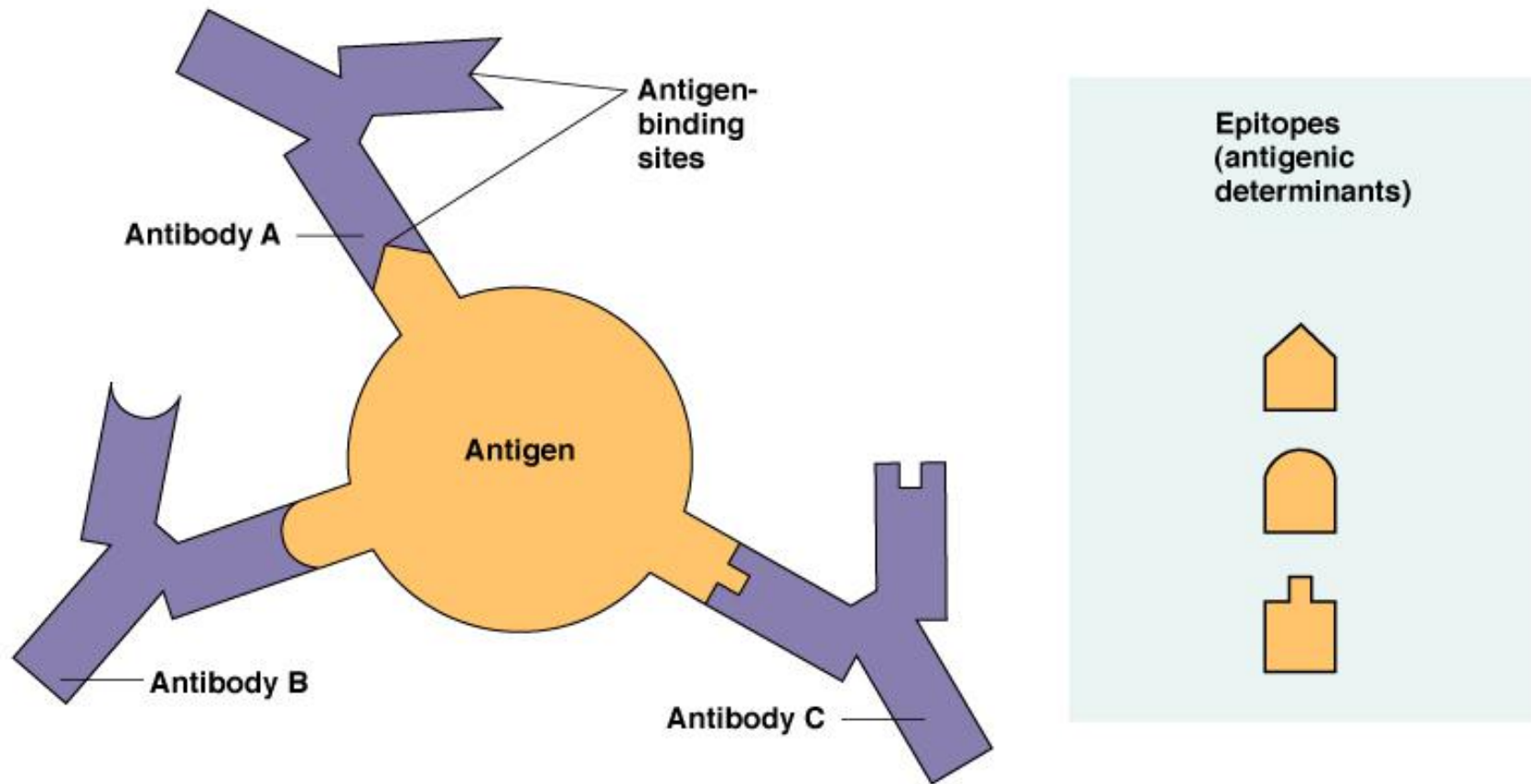
Two types:

- ✓ **Active immunization**= introduction of antigens and bacterial toxins treated by some chemicals to devoid disease born activity pathogens
- ✓ **passive immunization** = introduction of raid made antibodies obtained from blood of an actively immunized human being or animal

Serology

- Is examination of blood serum for antibodies using known antigens.
- serum is examined for antibodies as evidence of infection
- **Antibodies** :Proteins that recognize and bind to a particular antigen with very high *specificity*.
- **Antigens**: Some chemicals that create immune response. Most are proteins or large polysaccharides from a foreign organism

Antibody-antigen interaction



Enzyme-Linked Immunosorbent Assays (ELISA)

- ELISA techniques are becoming increasingly used in the diagnosis of microbial infections.
- They are **specific**, **sensitive**, and require only a small amount of specimen.
- **Sensitivity** is the ability of a test to correctly classify an animal as diseased
- **Specificity** is the ability of a test to correctly classify an animal as healthy.
- Large numbers of specimens can be tested at one time

Principles

- As its name suggests, the **enzyme linked-immunosorbent assay** uses an **enzyme system** to show the specific combination of an **antigen** with its **antibody**.
- An **enzyme conjugated** with an **antibody** reacts with a **colorless substrate** to generate a **colored** reaction product.
- A number of variations of ELISA have been developed

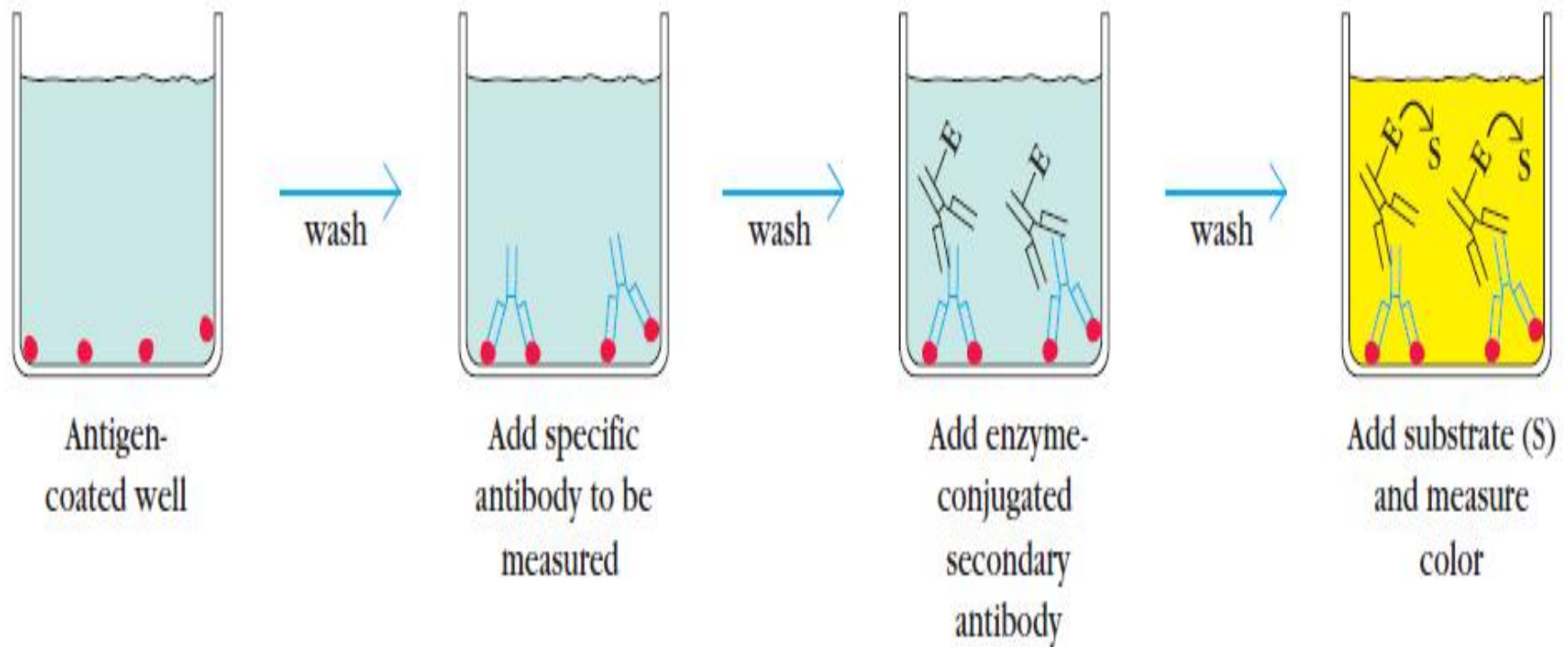
Indirect ELISA

- The most common form of ELISA is used to detect and measure specific antibodies.
- microwells in polystyrene plates are first filled with an antigen solution
- The serum under test is added to the wells. Any antibodies in the serum will bind to the antigen layer.

- After incubation and washing to remove unbound antibody, the presence of any bound antibodies can be detected by adding a solution containing an antiglobulin chemically linked to an enzyme.
- This labeled antiglobulin binds to the antibody and, following incubation and washing, can be detected and measured by adding a solution containing the enzyme substrate.

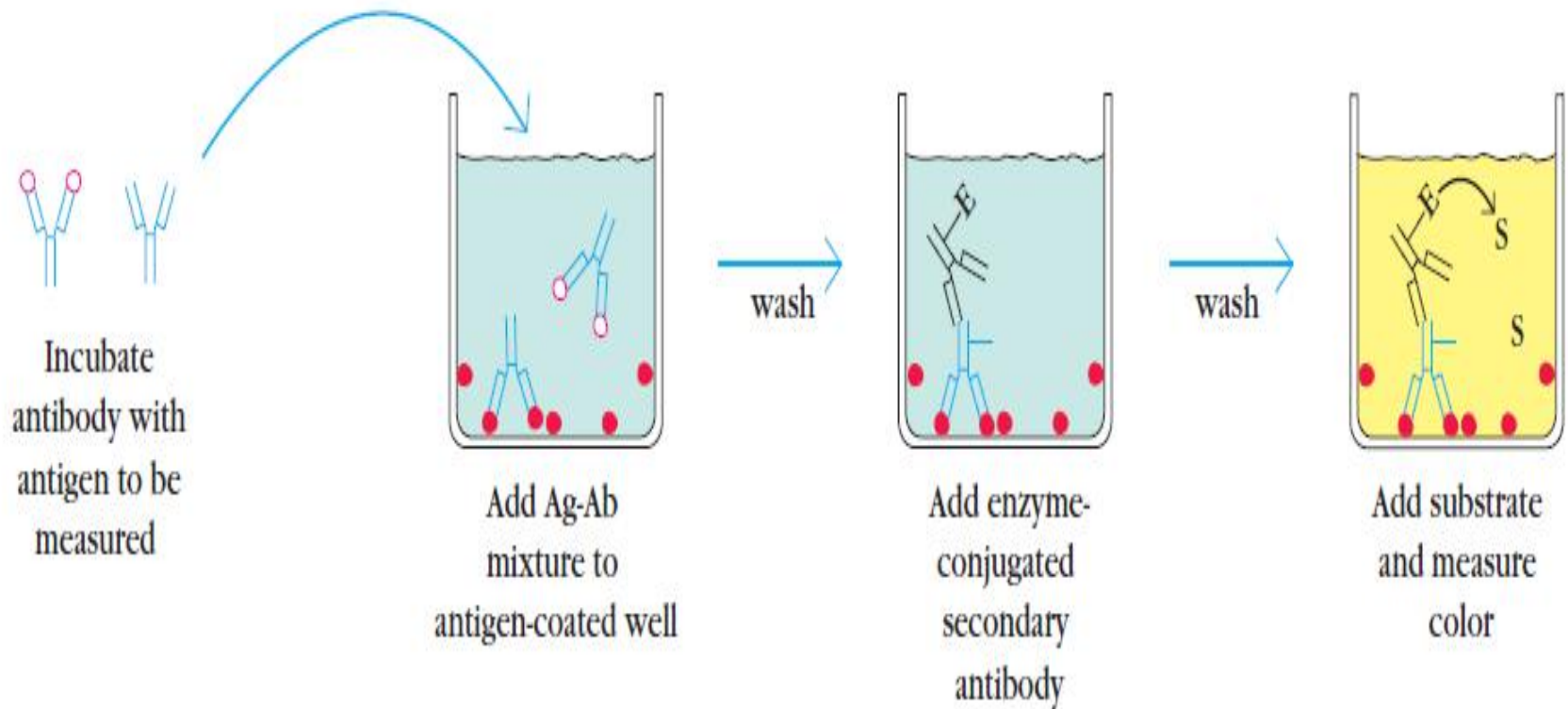
- The enzyme and substrate have been selected to ensure that a colored product develops in the tube.
- The intensity of the color that develops is therefore proportional to the amount of enzyme-linked antiglobulin that is bound, which in turn is proportional to the amount of antibody present in the serum under test.
- The color intensity may be estimated visually or, preferably, by spectrophotometry

Indirect ELISA

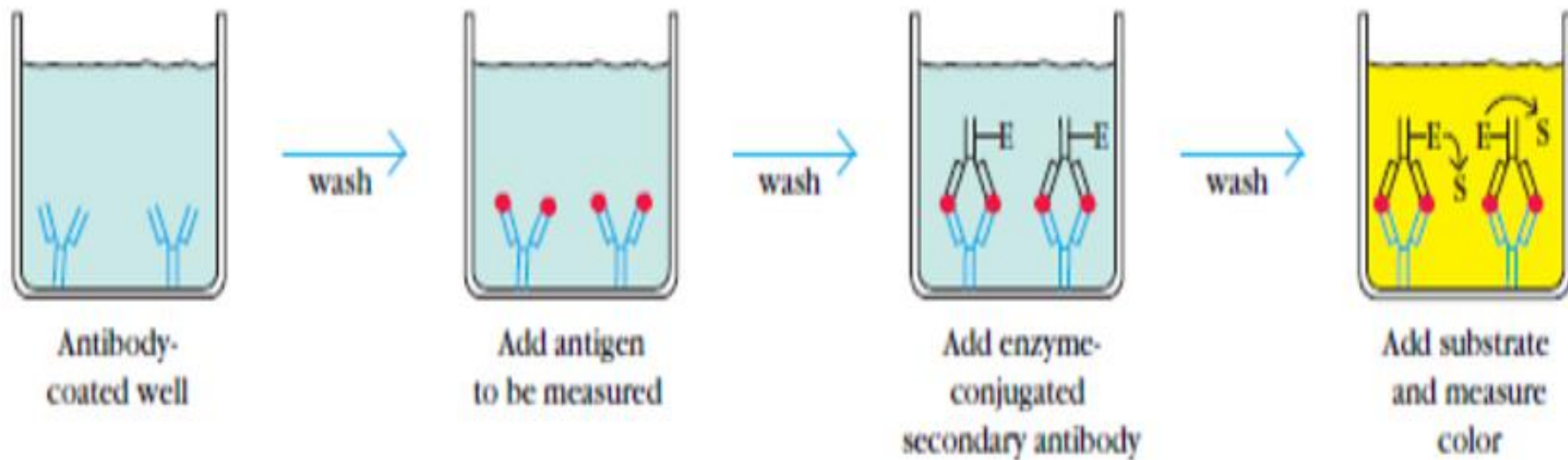


The indirect ELISA is used in the diagnosis of several parasitic infections and is being increasingly used in the diagnosis of bacterial, fungal and viral infections.

Competitive ELISA



- In the competitive assay, the higher the concentration of antigen in the original sample, the lower the absorbance. It can be used to measure viral antigens

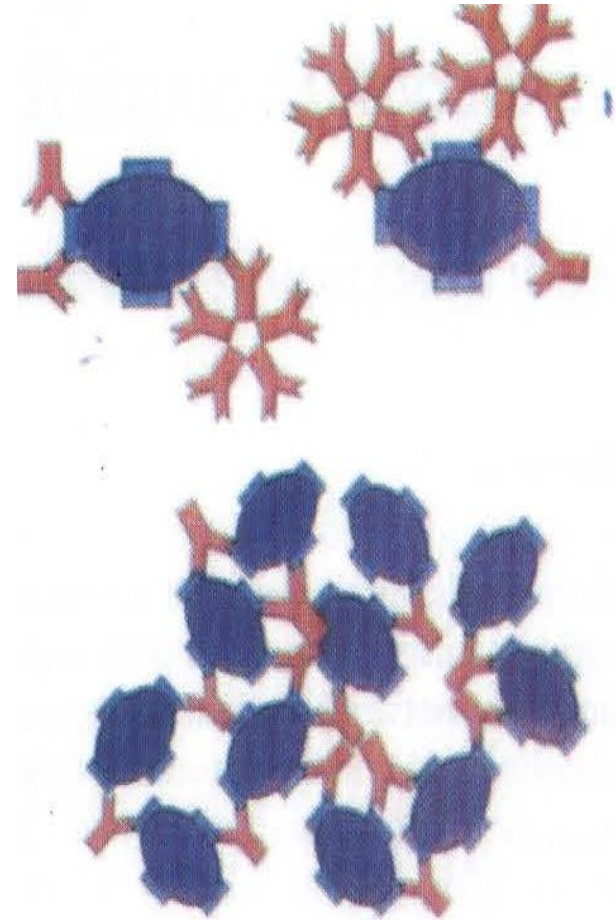


- Because this test involves the formation of antibody-antigen-antibody layers, it is called **sandwich ELISA**.
- It is used to detect circulating virus in blood from cats with feline leukemia.

Agglutination laboratory test

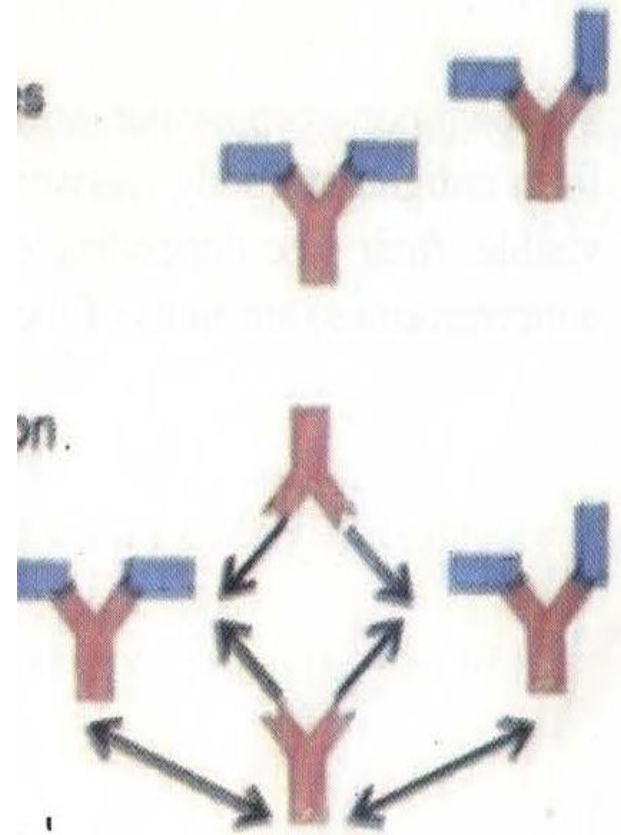
- **Agglutination reaction** is a reaction between antibodies and corpuscular antigens

E.g . Rose Bengal plate agglutination test using *Brucella abortus* cells for detection of specific antibodies



Precipitation serological test




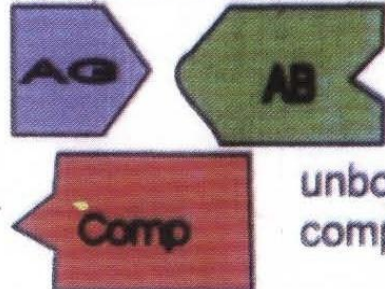
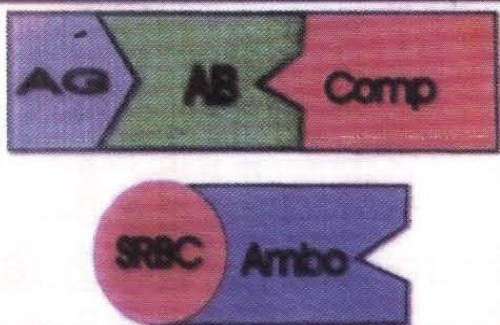

- **Precipitation** test is a reaction between soluble antigen & corresponding antibodies
- ✓ Formation soluble Ab-Ag complex
- ✓ Aggregations of complex
- ✓ If aggregation grow large
- ✓ Become insoluble & precipitate.
- ✓ this takes place in liquid and semi -liquid medium
- e.g. **Ascoli test** for detection of soluble *Bacillus anthracis* antigen



Complement fixation test

- ✓ **Complement is one of nonspecific defense mechanism.**
- ✓ **Comprises more than 20 serum proteins**
- ✓ **The proteins are activated in reaction cascade & aggregates to a membrane attack complex, which make a pore in the cell membrane of corpuscular (cellular) antigens.**

CFT principle

Positive sample	Reaction	Negative sample
 <p>Formation of immune complexes</p>	<p>Test reaction :</p> <p>Addition of serum sample to test antigen</p>	 <p>unbound antibodies</p>
 <p>Fixation of complement by immune complexes</p>	<p>Addition of complement</p>	 <p>unbound complement</p>
	<p>Indicator reaction :</p> <p>Addition of SRBC and Amboceptor</p>	
<p>⇒ Sedimentation of SRBC</p>	<p>Result</p>	<p>⇒ Lysis of SRBC by activated complement</p>

Fluorescent antibody test

- ✓ Drops of appropriately diluted fluorescein labeled antibody(conjugate) are applied to the
- ✓ Antigen on slides
- ✓ then incubate in a humid chamber
- ✓ After incubation, the drops of conjugate are shaken off & the slides washed
- ✓ Buffer glycerol mounting fluid is added to the last sediment &
- ✓ The immune complexes can be identified under an UV- microscope

Immunization: The process of producing a state of immunity in a subject

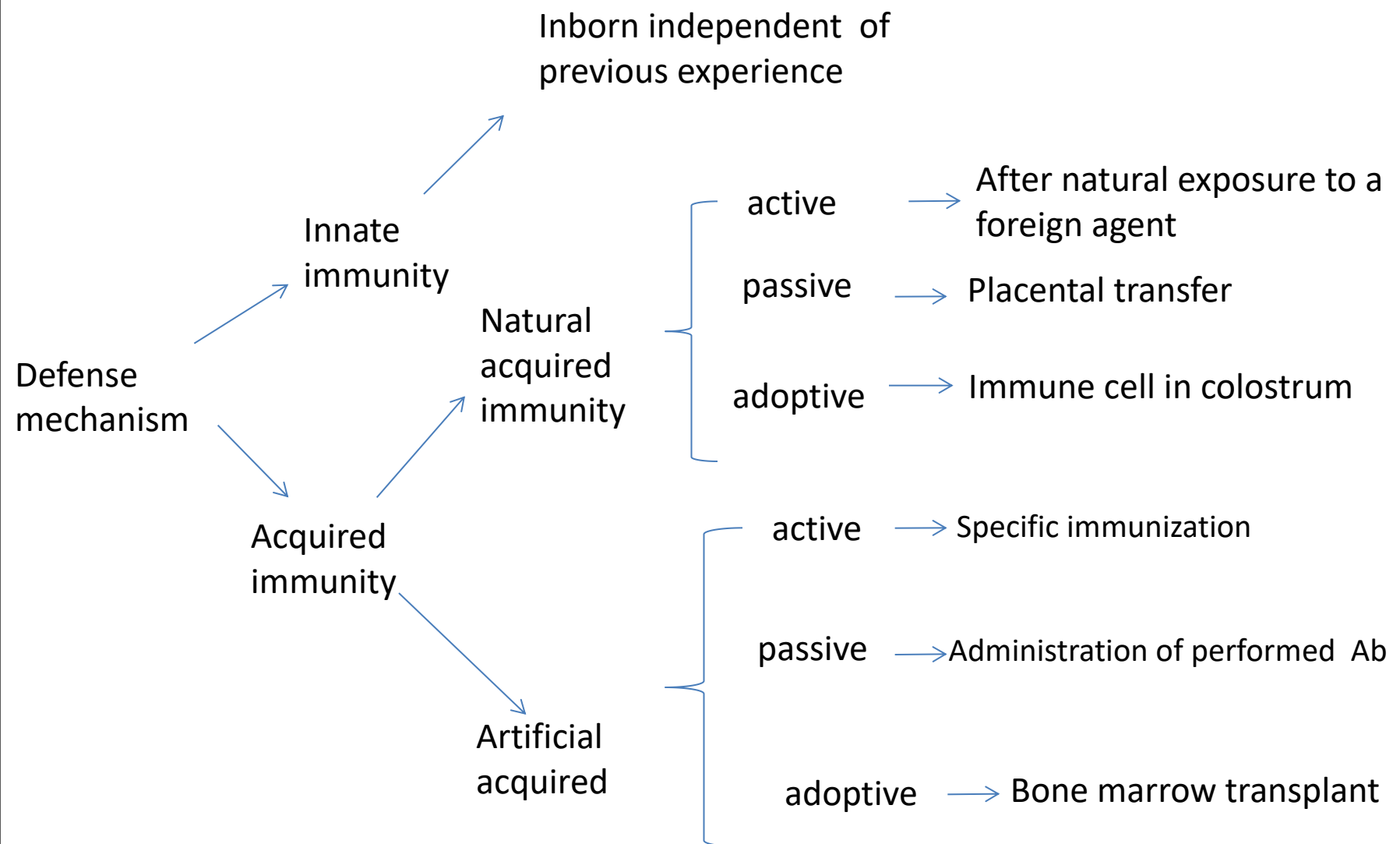
Types of immunization procedures

- There are two basic methods by which any animal may be made immune to an infectious disease:
 - a) Passive immunization**
 - b) Active immunization**

A) Passive immunization

Achieved by:

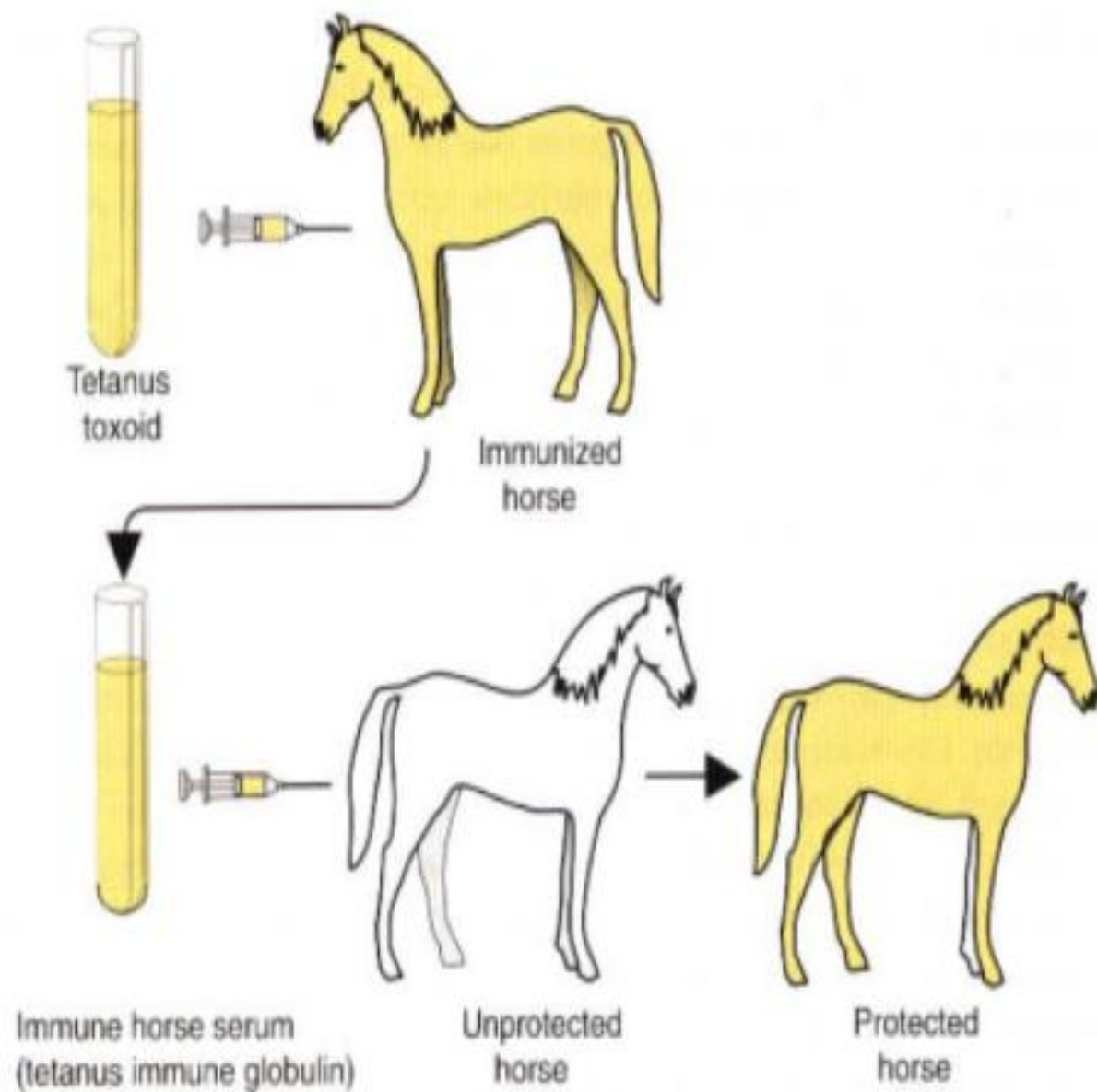
- Transfer of preformed antibodies from dam to the developing fetus across placenta
- Transfer of maternal antibodies to newborns through colostrum



→ Injecting a recipient animal with preformed antibodies (immunoglobulins) in donor animals by active immunization

e.g. anthrax (cattle), distemper (dog), panleukopenia (cat),
Tetanus (horses)

Figure 1-6. Transfer of immunity to tetanus by means of serum derived from an immunized horse. This clearly demonstrates that antibodies in serum are sufficient to confer immunity to tetanus.



Advantage of passive immunity

- Confer immediate protection

Disadvantage

- No memory response and the protection provided is short-lived
- Elicit an immune response when given to other species of animal (immunoglobulins, mAbs)
- Transmission of **infections** from donor to recipient

B) Active immunization

- Achieved by **natural infection** with a **microorganism**, or
- **Acquired artificially** by administration of a **vaccine**

Advantage

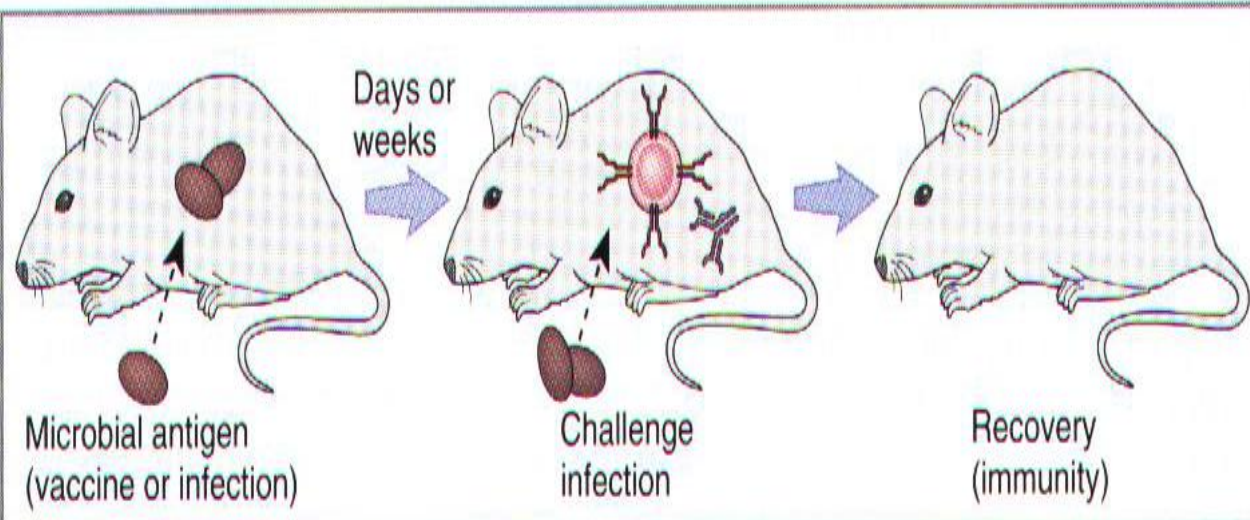
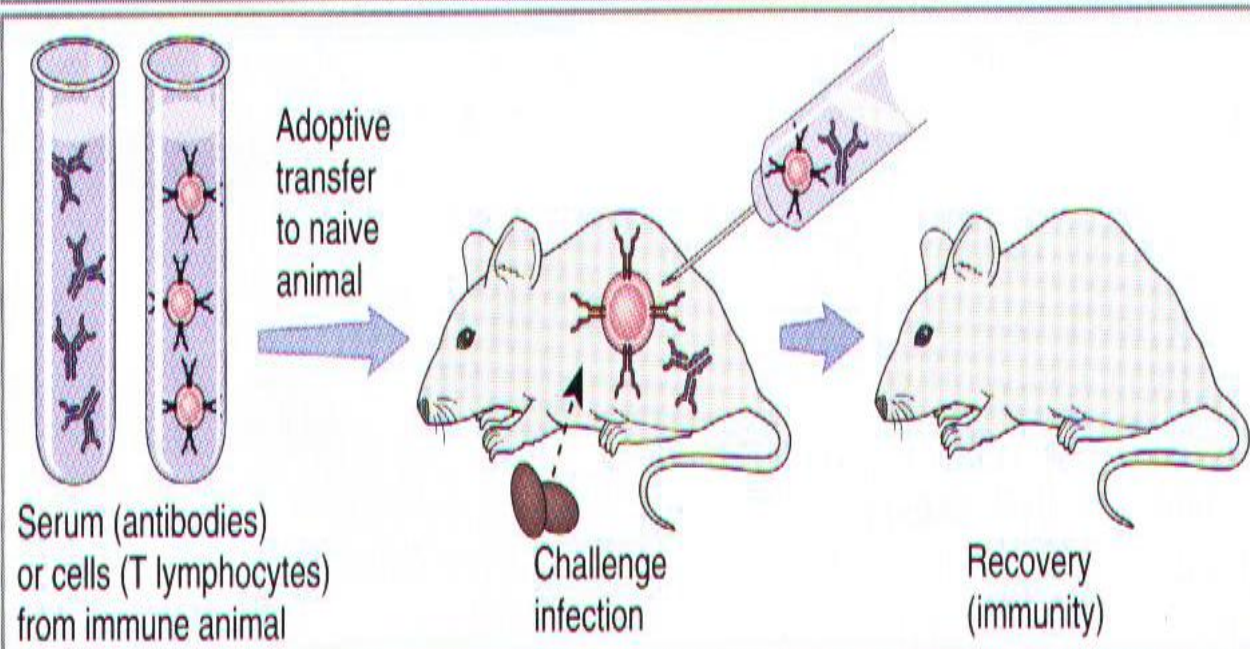
- Provide long-lasting immunity
- Capable of re-stimulation by repeated injections of antigen or exposure to infection

Disadvantage

- Protection is not conferred immediately

Passive Vs Active Immunization

Feature	Passive	Active
Protection	Immediate	Requires 7-10 days
Duration	Days to months	Years
Host immune system	Not involved	Required
Elements of response	Anti-pathogen Ab only	Anti-pathogen Ab, CTLs, Th cells
Memory	No	Yes
Uses	Immunocompromised individual unvaccinated individual where no vaccine exist	Immunocompetent individual individuals not naturally exposed to pathogen where a safe, efficacious vaccine exist

			Specificity	Memory	
Active immunity	 <p>Microbial antigen (vaccine or infection)</p> <p>Days or weeks</p> <p>Challenge infection</p> <p>Recovery (immunity)</p>			Yes	Yes
Passive immunity	 <p>Serum (antibodies) or cells (T lymphocytes) from immune animal</p> <p>Adoptive transfer to naive animal</p> <p>Challenge infection</p> <p>Recovery (immunity)</p>			Yes	No

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So what is vaccine?

Vaccine: A preparation of immunogenic material used to induce immunity against pathogenic organisms

Vaccination: Intentional administration of a harmless or less harmful form of a pathogen to induce a specific immune response that protects the individual against later exposure to the pathogen

THANK YOU!!!