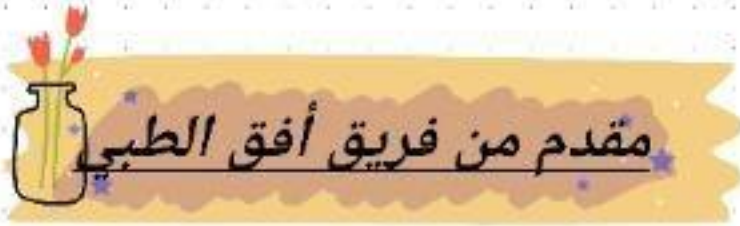
مقدم من فريق أفق الطبي


جامعة الأزهر - غزة
AL-AZHAR UNIVERSITY - GAZA
1412-1991 - ١٤١٢-١٩٩١


حركة الشبيبة الفتحاوية
جامعة الأزهر
Fateh Youth Movement

General Microbiology

Bacteria Compared with Other Microorganisms

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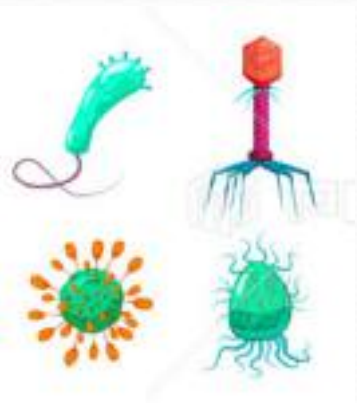


Introduction

- **Biology:** The science of living things.
- **Microbiology:** A branch of biology dealing with microscopic forms of life as bacteria, protozoa, viruses and fungi.
- **Branches of Microbiology:**
- Bacteriology, Parasitology, mycology and virology.

Microbes that cause infectious diseases

- The agents of human infectious diseases belong to five major groups of organisms: bacteria, fungi, protozoa, helminths, and viruses.
- Bacteria belong to the prokaryote kingdom,
- Fungi (yeasts and molds) belong to the kingdom of fungi
- protozoa are members of the kingdom of protists.
- Helminths (worms) are classified in the animal kingdom
- Protists and fungi are distinguished from animals and plants by being either unicellular or relatively simple multicellular organisms.
- Helminths are complex multicellular organisms.

Microbes that cause infectious diseases

- The helminths and the protozoa are commonly called parasites.
- Viruses are quite distinct from other organisms—they are not cells but can replicate only within cells.

Important features of microbes

This distinction is based primarily on three criteria:

(1) **Structure.**

(2) Method of replication.

(3) Nature of the nucleic acid.

(1) Structure

- Cells have a nucleus or nucleoid which contains DNA; this is surrounded by cytoplasm, within which proteins are synthesized and energy is generated.
- Viruses have an inner core of genetic material (either DNA or RNA) but no cytoplasm, and so they depend on host cells to provide the machinery for protein synthesis and energy generation.

(2) Method of replication.

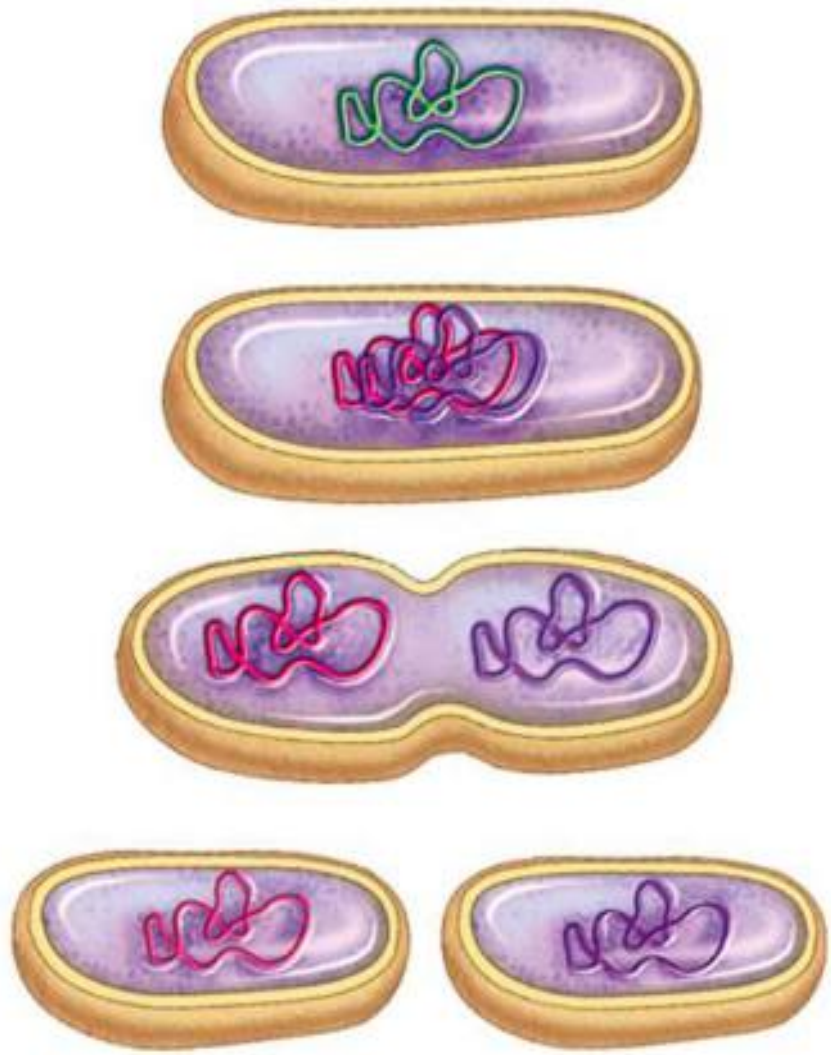
- Cells replicate either by binary fission or by mitosis, during which one parent cell divides to make two progeny cells while retaining its cellular structure.
- Prokaryotic cells (e.g., bacteria) replicate by binary fission, whereas eukaryotic cells replicate by mitosis.
- In contrast, viruses disassemble, produce many copies of their nucleic acid and protein, and then reassemble into multiple progeny viruses.
- Viruses must replicate within host cells because they lack protein-synthesizing and energy-generating systems.
- With the exception of rickettsiae and chlamydiae, which also require living host cells for growth, bacteria can replicate extracellularly.

(3) Nature of the nucleic acid.

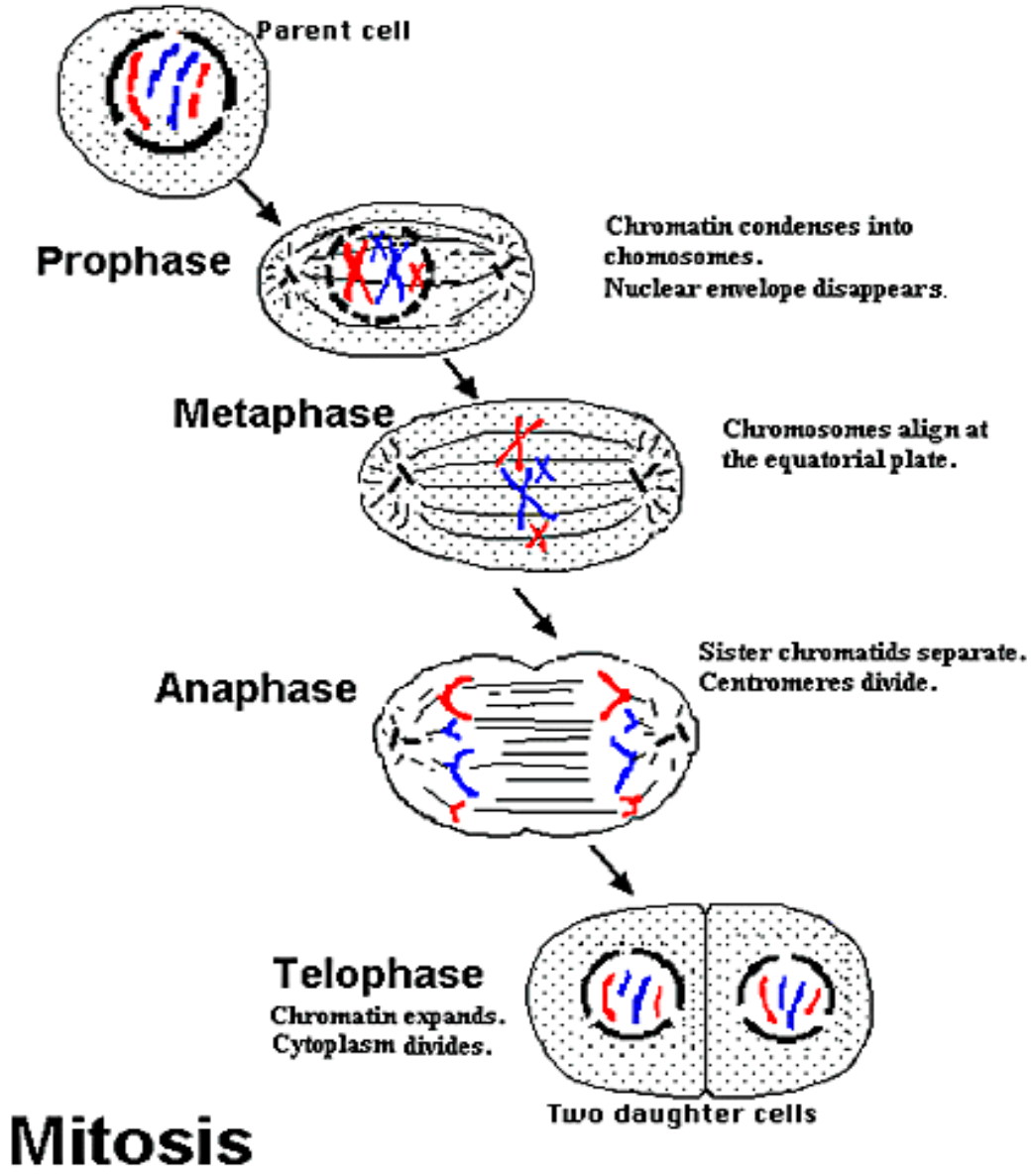
- Cells contain both DNA and RNA, whereas viruses contain either DNA or RNA, but not both.

Eukaryotes & prokaryotes

- Eukaryotic cell has a true nucleus with multiple chromosomes surrounded by a nuclear membrane and uses a mitotic apparatus to ensure equal allocation of the chromosomes to progeny cells.
- The nucleoid of a prokaryotic cell consists of a single circular molecule of loosely organized DNA, lacking a nuclear membrane and mitotic apparatus
- Eukaryotic cells contain organelles, such as mitochondria and lysosomes, and larger (80S) ribosomes, whereas prokaryotes contain no organelles and smaller (70S) ribosomes.
- Most prokaryotes have a rigid external cell wall that contains peptidoglycan, a polymer of amino acids and sugars, as its unique structural component.



Binary Fission


Parent cell
Prophase
Chromatin condenses into chomosomes.
Nuclear envelope disappears.
Metaphase
Chromosomes align at the equatorial plate.
Anaphase
Sister chromatids separate.
Centromeres divide.
Telophase
Chromatin expands.
Cytoplasm divides.
Two daughter cells

Mitosis

Eukaryotes & Prokaryotes

- Eukaryotes, do not contain peptidoglycan.
- Either they are bound by a flexible cell membrane, or in the case of fungi, they have a rigid cell wall with chitin, a homopolymer of N-acetylglucosamine, typically forming the framework.
- The eukaryotic cell membrane contains **sterols**, whereas no prokaryote, except the wall-less *Mycoplasma*, has sterols in its membranes

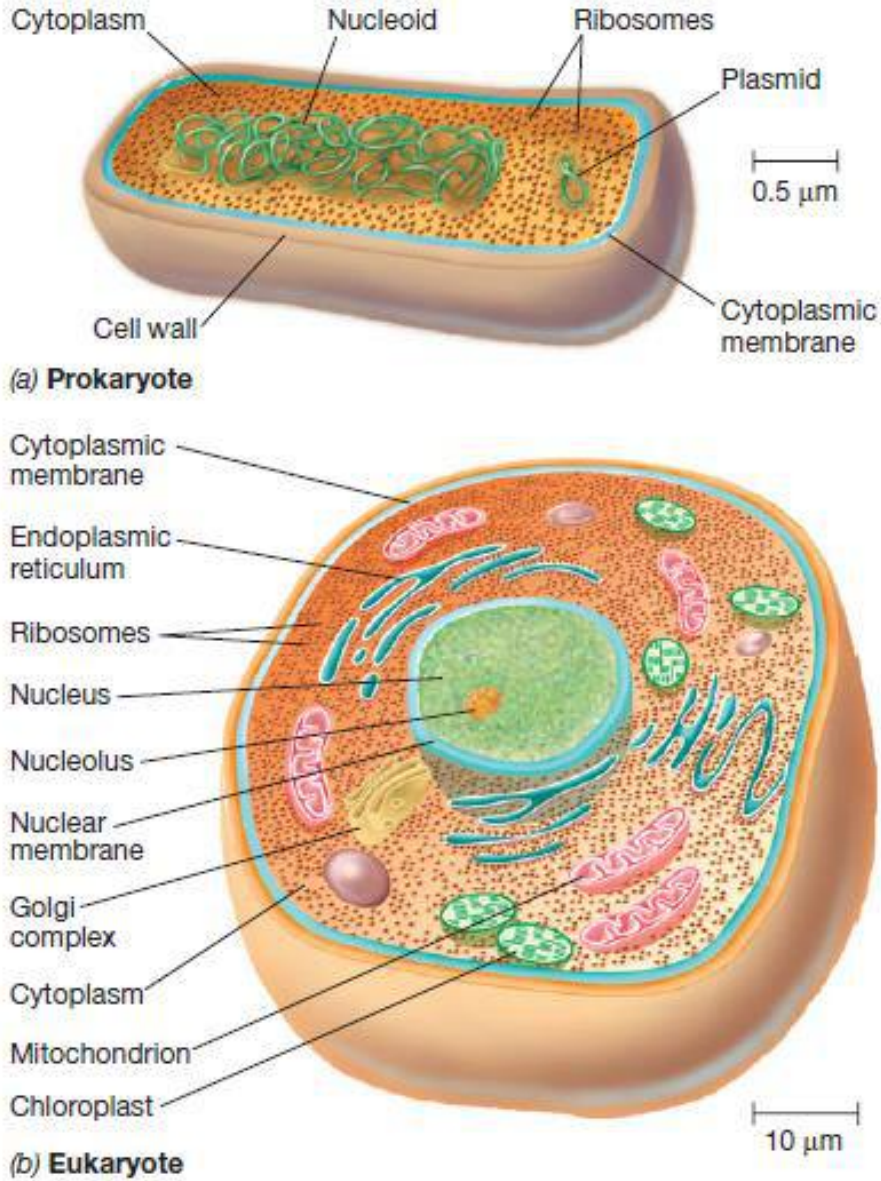


Figure 2.11 Internal structure of cells. Note differences in scale and internal structure between the prokaryotic and eukaryotic cells.

TABLE 1–3 Characteristics of Prokaryotic and Eukaryotic Cells

Characteristic	Prokaryotic Bacterial Cells	Eukaryotic Human Cells
DNA within a nuclear membrane	No	Yes
Mitotic division	No	Yes
DNA associated with histones	No	Yes
Chromosome number	One	More than one
Membrane-bound organelles, such as mitochondria and lysosomes	No	Yes
Size of ribosome	70S	80S
Cell wall containing peptidoglycan	Yes	No

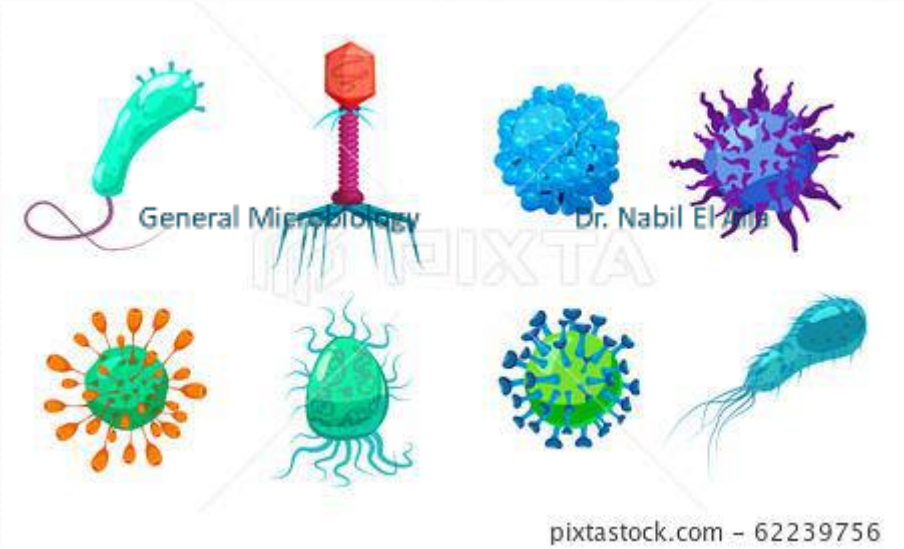
TABLE 1–2 Comparison of Medically Important Organisms

Characteristic	Viruses	Bacteria	Fungi	Protozoa and Helminths
Cells	No	Yes	Yes	Yes
Approximate diameter (μm)[1]	0.02–0.2	1–5	3–10 (yeasts)	15–25 (trophozoites)
Nucleic acid	Either DNA or RNA	Both DNA and RNA	Both DNA and RNA	Both DNA and RNA
Type of nucleus	None	Prokaryotic	Eukaryotic	Eukaryotic
Ribosomes	Absent	70S	80S	80S
Mitochondria	Absent	Absent	Present	Present
Nature of outer surface	Protein capsid and lipoprotein envelope	Rigid wall containing peptidoglycan	Rigid wall containing chitin	Flexible membrane
Motility	None	Some	None	Most
Method of replication	Not binary fission	Binary fission	Budding or mitosis[2]	Mitosis[3]

TABLE 1–1 Biologic Relationships of Pathogenic Microorganisms

Kingdom	Pathogenic Microorganisms	Type of Cells
Animal	Helminths (worms)	Eukaryotic
Protists	Protozoa	Eukaryotic
Fungi	Fungi (yeasts and molds)	Eukaryotic
Prokaryote	Bacteria Viruses	Prokaryotic Noncellular

End of Lecture



General Microbiology

Structure of Bacterial Cell

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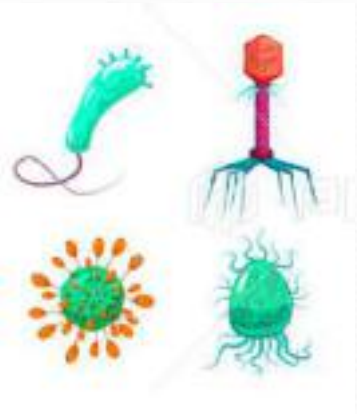


Shape & size of bacteria

- Bacteria are classified by shape into three basic groups:
- cocci,
- certain cocci occur in pairs (diplococci), some in chains (streptococci), and others in grapelike clusters (staphylococci).
- bacilli,
- Spirochetes
- The arrangement of rods and spirochetes is medically less
- important

Shape & size of bacteria

- Bacteria not only vary in size but also in shape, the more typical forms are:
- Cocci (spherical shape):
- a. Diplococci b. Streptococci C. Staphylococci
- Bacilli (rod shaped):

a- Gram negative bacilli (e.g. *Salmonella* and *Shigella*)

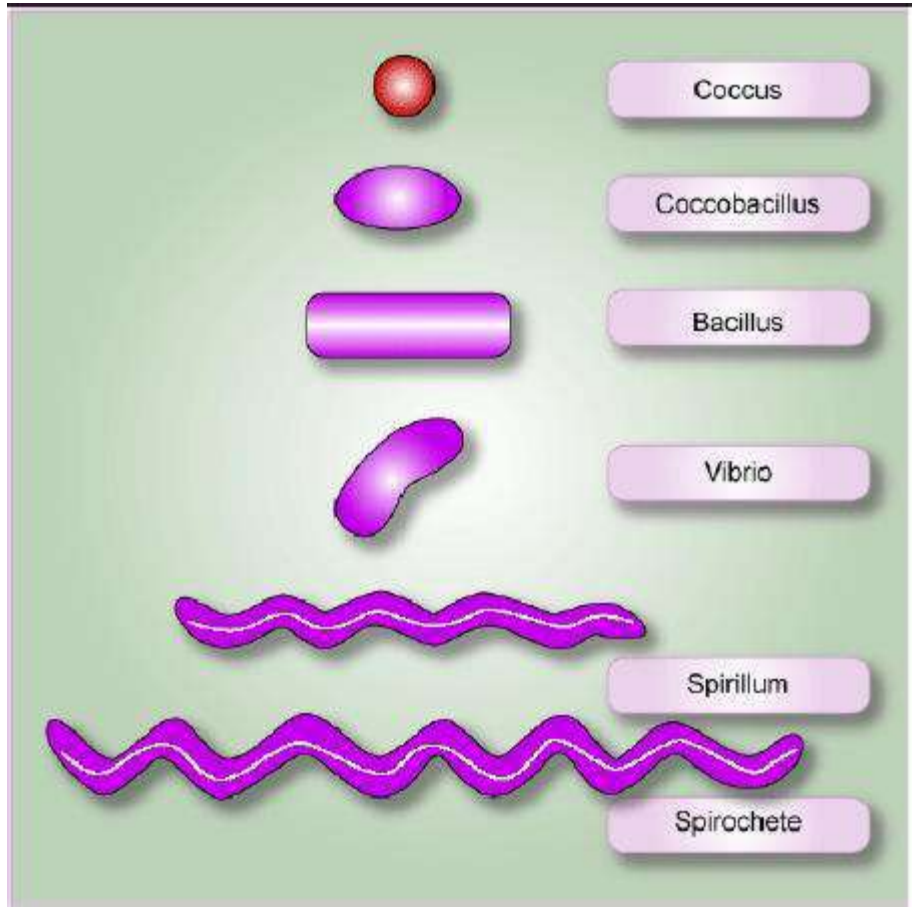
b- Gram positive bacilli

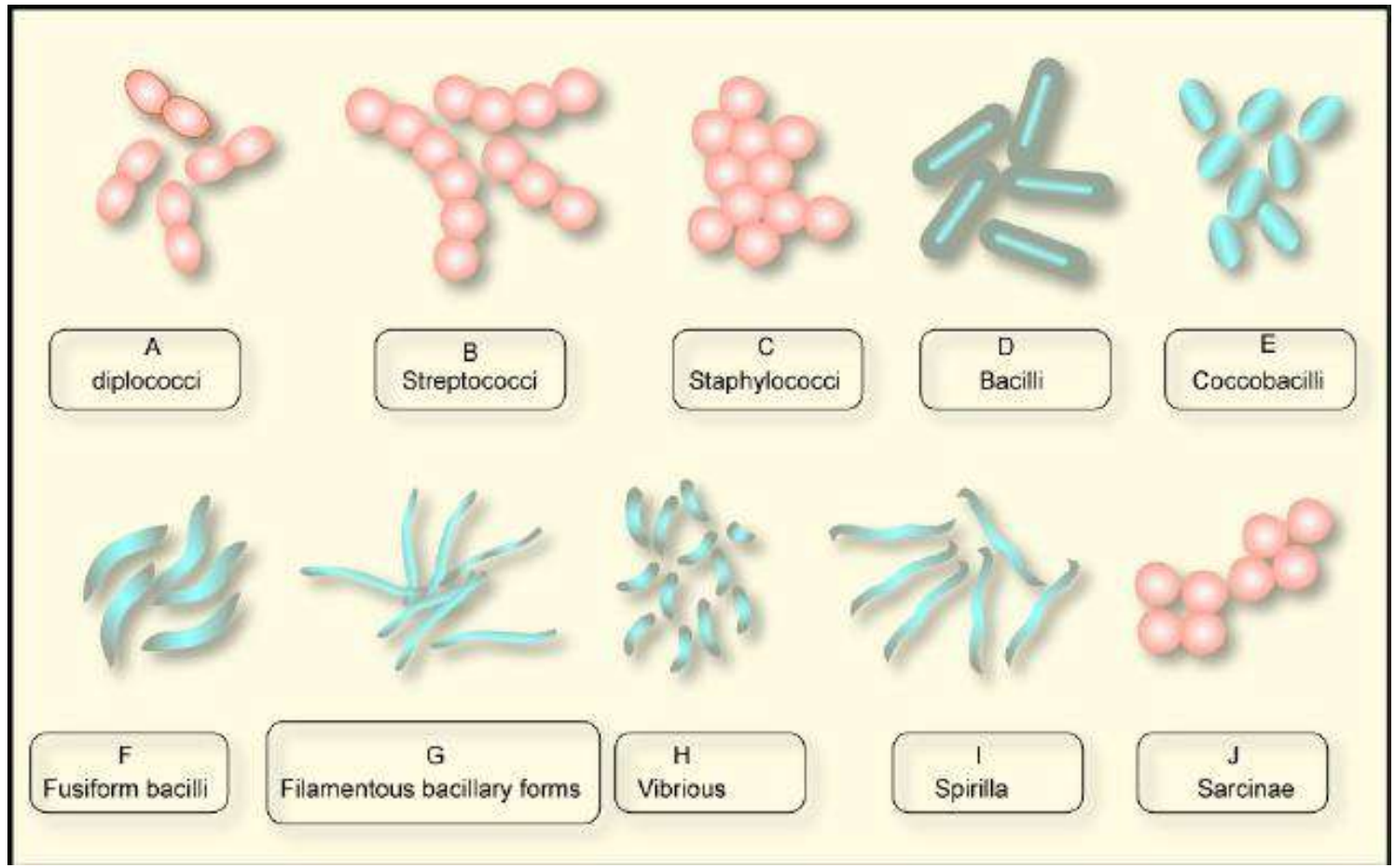
- Sporing (aerobic and anaerobic)
- Non sporing (e.g. *Corynebacterium)*

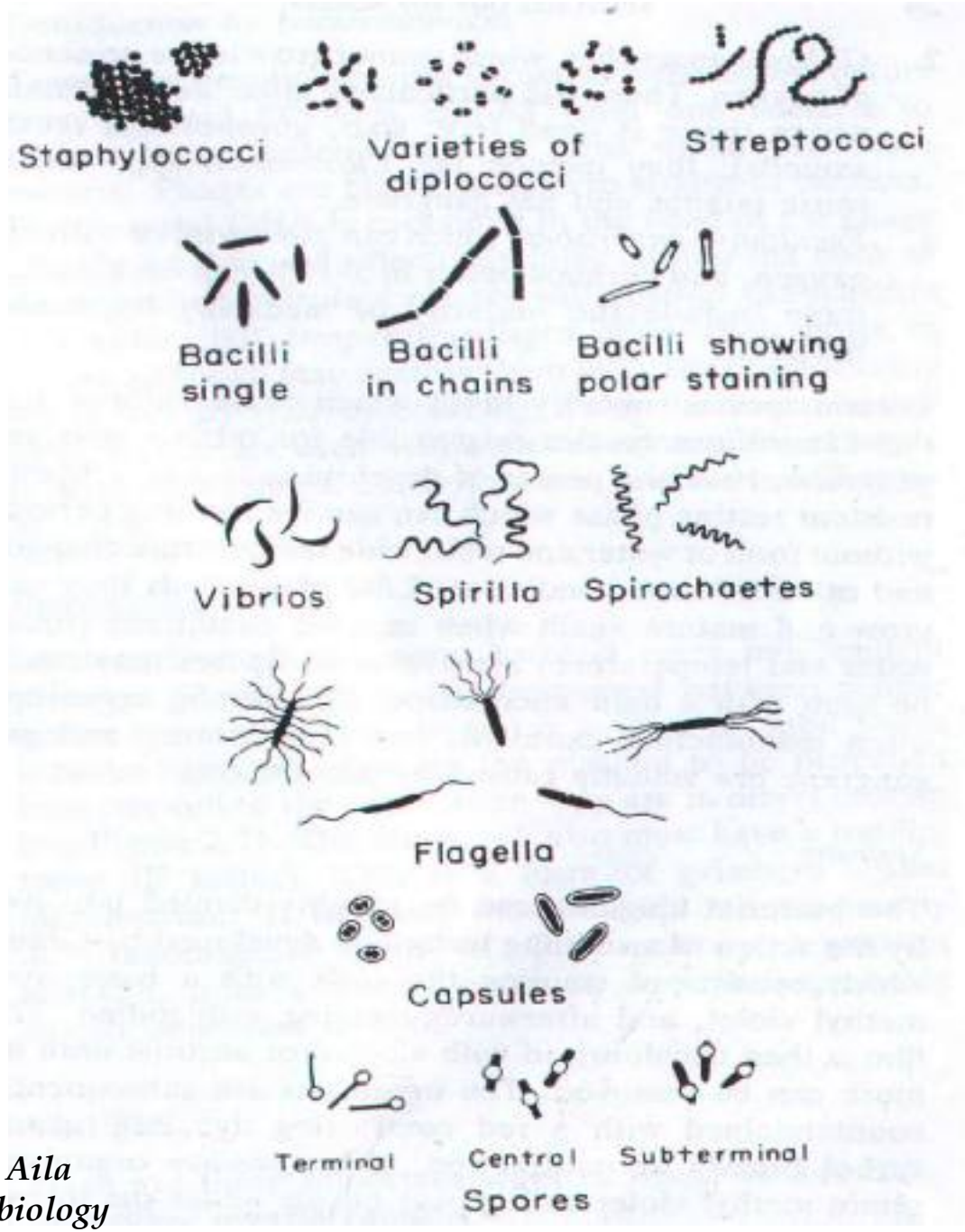
c- Acid fast bacilli (e.g. *Mycobacterium*)

Gross Morphology of Bacteria

- Vibrios (comma shaped) e.g. *Vibrio cholera*
- Spiral e.g. Spirochetes.
- Filamentous e.g. Actinomyces.
- Fusiforms (spindle shaped).


Coccus
Coccobacillus
Bacillus
Vibrio
Spirillum
Spirochete


A
diplococci
B
Streptococci
C
Staphylococci
D
Bacilli
E
Coccobacilli
F
Fusiform bacilli
G
Filamentous bacillary forms
H
Vibrious
I
Spirilla
J
Sarcinae


Staphylococci
Varieties of diplococci
Streptococci
Bacilli single
Bacilli in chains
Bacilli showing polar staining
Vibrios
Spirilla
Spirochaetes
Flagella
Capsules
Terminal
Central
Subterminal
Spores

Shape & size of bacteria

- Bacteria range in size from about 0.2 to 5 µm
- The smallest bacteria (Mycoplasma) are about the same size as the largest viruses (poxviruses)
- The longest bacteria rods are the size of some yeasts and human red blood cells (7 µm).

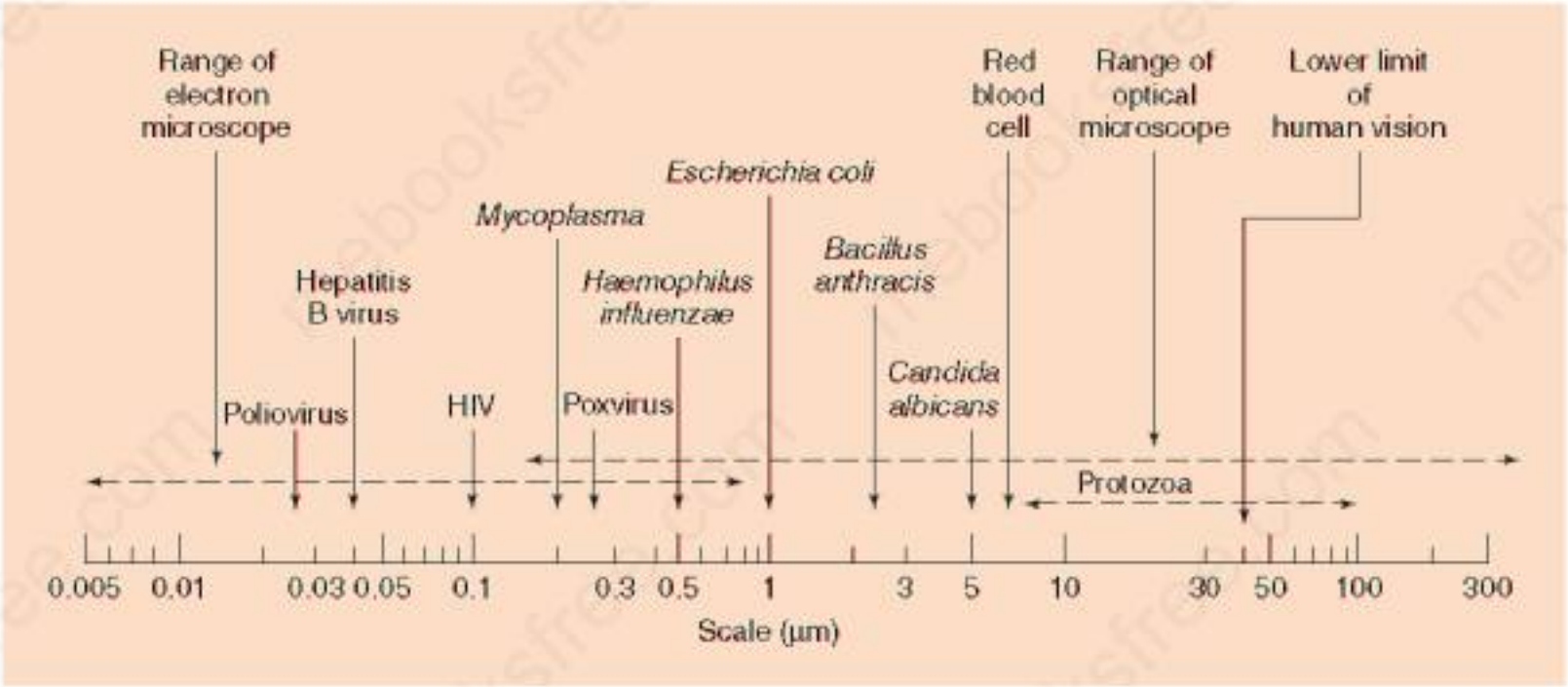


FIGURE 2–2 Sizes of representative bacteria, viruses, yeasts, protozoa, and human red cells. The bacteria range in size from *Mycoplasma*,

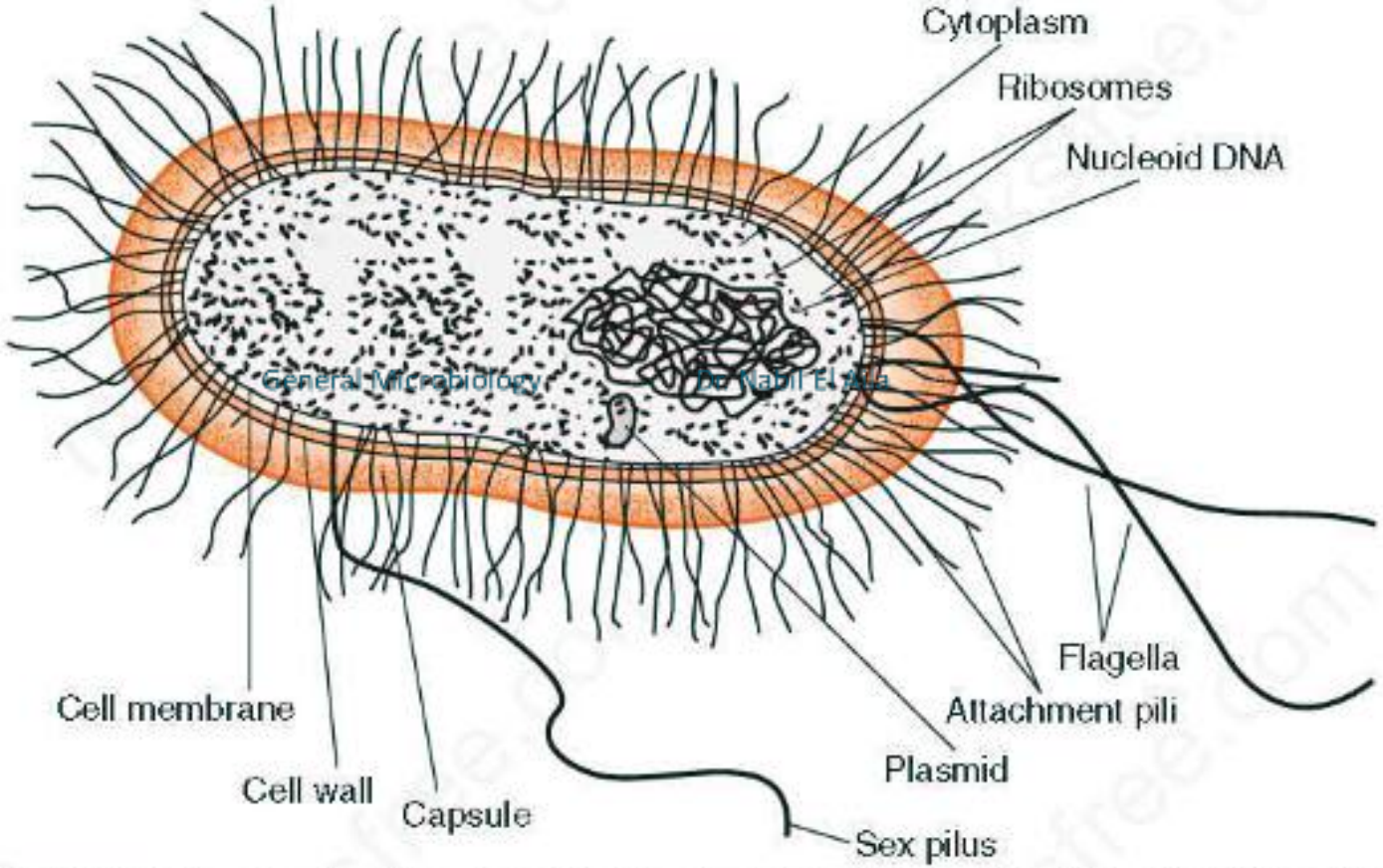


FIGURE 2–3 Bacterial structure. (Reproduced with permission from Ryan K et al. *Sherris Medical Microbiology*. 4th ed. Copyright 2004, McGraw-Hill.)

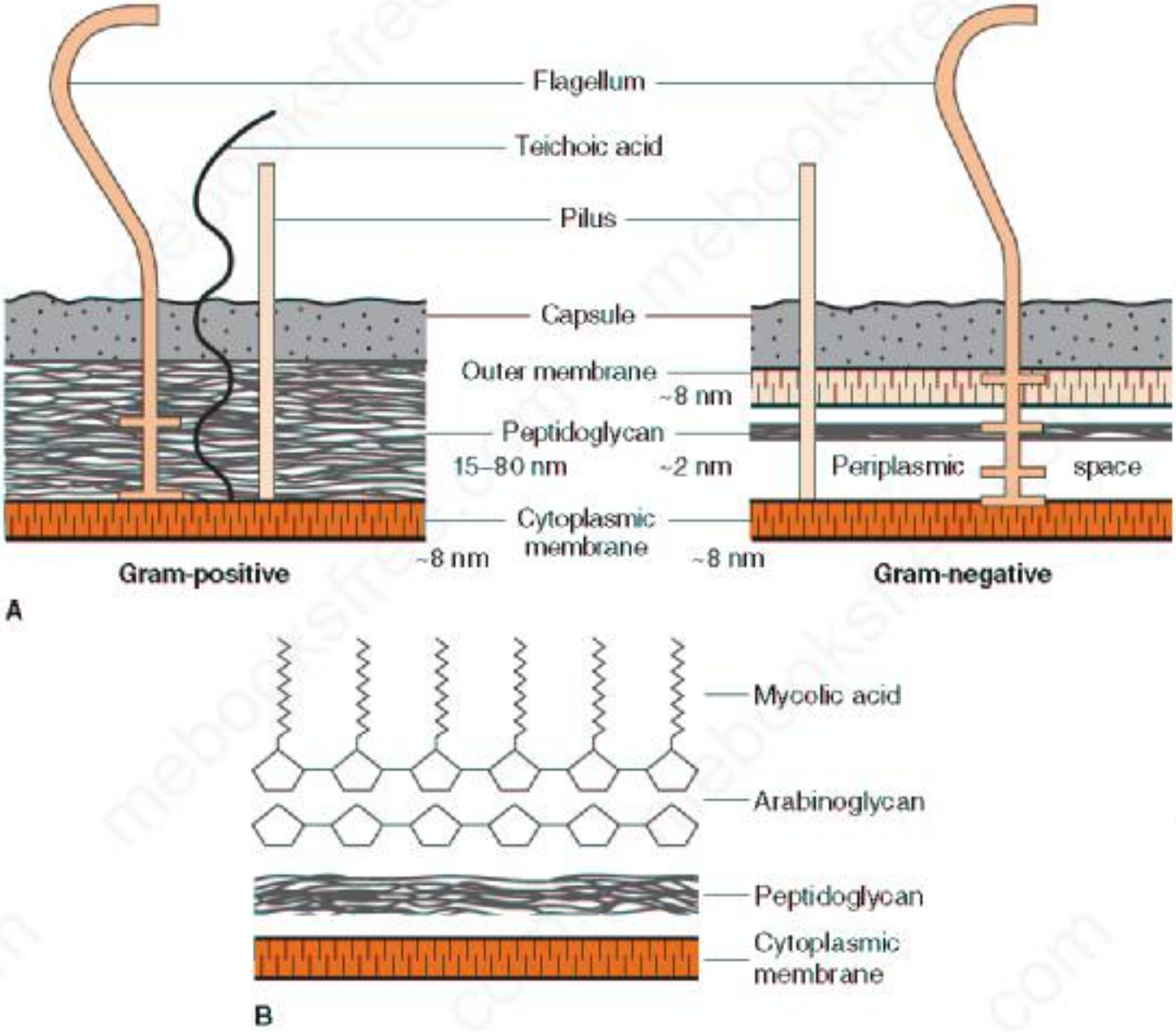
STRUCTURE OF BACTERIA

Cell Wall

- The cell wall is the outermost component common to all bacteria (except Mycoplasma species, which are bounded by a cell membrane, not a cell wall).
- Some bacteria have surface features external to the cell wall, such as a capsule, flagella, and pili

Cell Walls of Gram-Positive and Gram-Negative Bacteria

- The peptidoglycan layer is much thicker in gram positive than in gram-negative bacteria.
- Many gram-positive bacteria also have fibers of teichoic acid that protrude outside the peptidoglycan, whereas gram-negative bacteria do not have teichoic acids.
- Gram-negative bacteria have a complex outer layer consisting of lipopolysaccharide, lipoprotein, and phospholipid.
- Lying between the outer-membrane layer and the cytoplasmic membrane in gram-negative bacteria is the periplasmic space, which is the site, in some species, of enzymes called β-lactamases that degrade penicillins and other β-lactam drugs


Flagellum
Teichoic acid
Pilus
Capsule
Outer membrane
~8 nm
Peptidoglycan
15–80 nm
~2 nm
Periplasmic space
Cytoplasmic membrane
~8 nm
~8 nm
Gram-positive
Gram-negative
A
Mycolic acid
Arabinoglycan
Peptidoglycan
Cytoplasmic membrane
B

Cell walls of bacteria

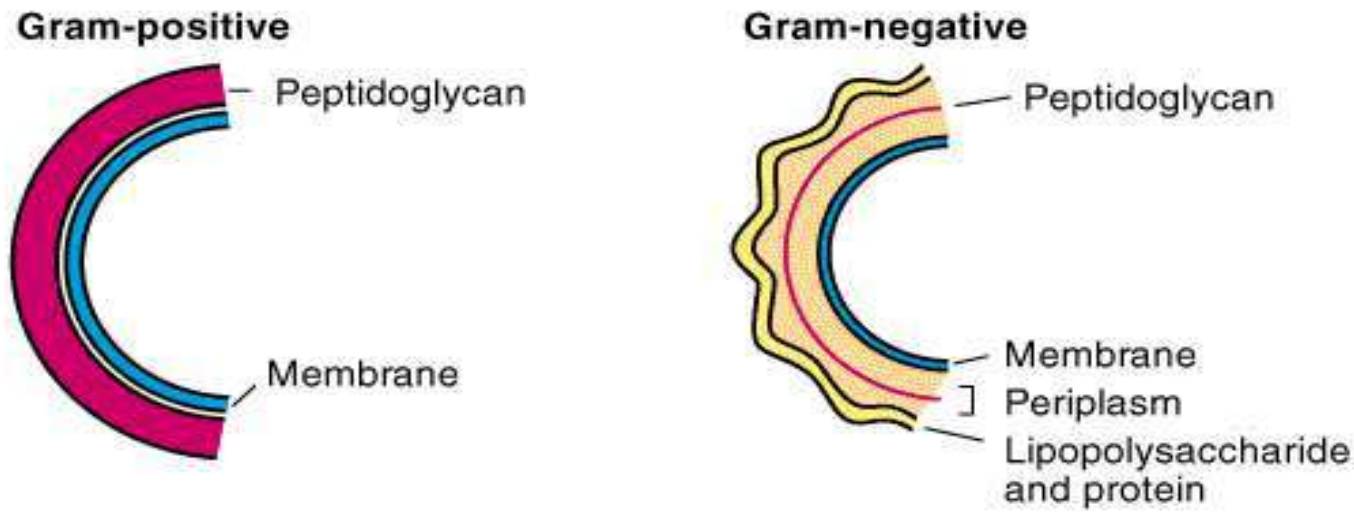


TABLE 2–2 Comparison of Cell Walls of Gram-Positive and Gram-Negative Bacteria

Component	Gram-Positive Cells	Gram-Negative Cells
Peptidoglycan	Thicker; multilayer	Thinner; single layer
Teichoic acids	Yes	No
Lipopolysaccharide (endotoxin)	No	Yes

TABLE 2–3 Medically Important Bacteria That Cannot Be Seen in the Gram Stain

Name	Reason	Alternative Microscopic Approach
Mycobacteria, including *M. tuberculosis*	Too much lipid in cell wall so dye cannot penetrate	Acid-fast stain
Treponema pallidum	Too thin to see	Dark-field microscopy or fluorescent antibody
Mycoplasma pneumoniae	No cell wall; very small	None
Legionella pneumophila	Poor uptake of red counterstain	Prolong time of counterstain
Chlamydiae, including *C. trachomatis*	Intracellular; very small	Inclusion bodies in cytoplasm
Rickettsiae	Intracellular; very small	Giemsa or other tissue stains

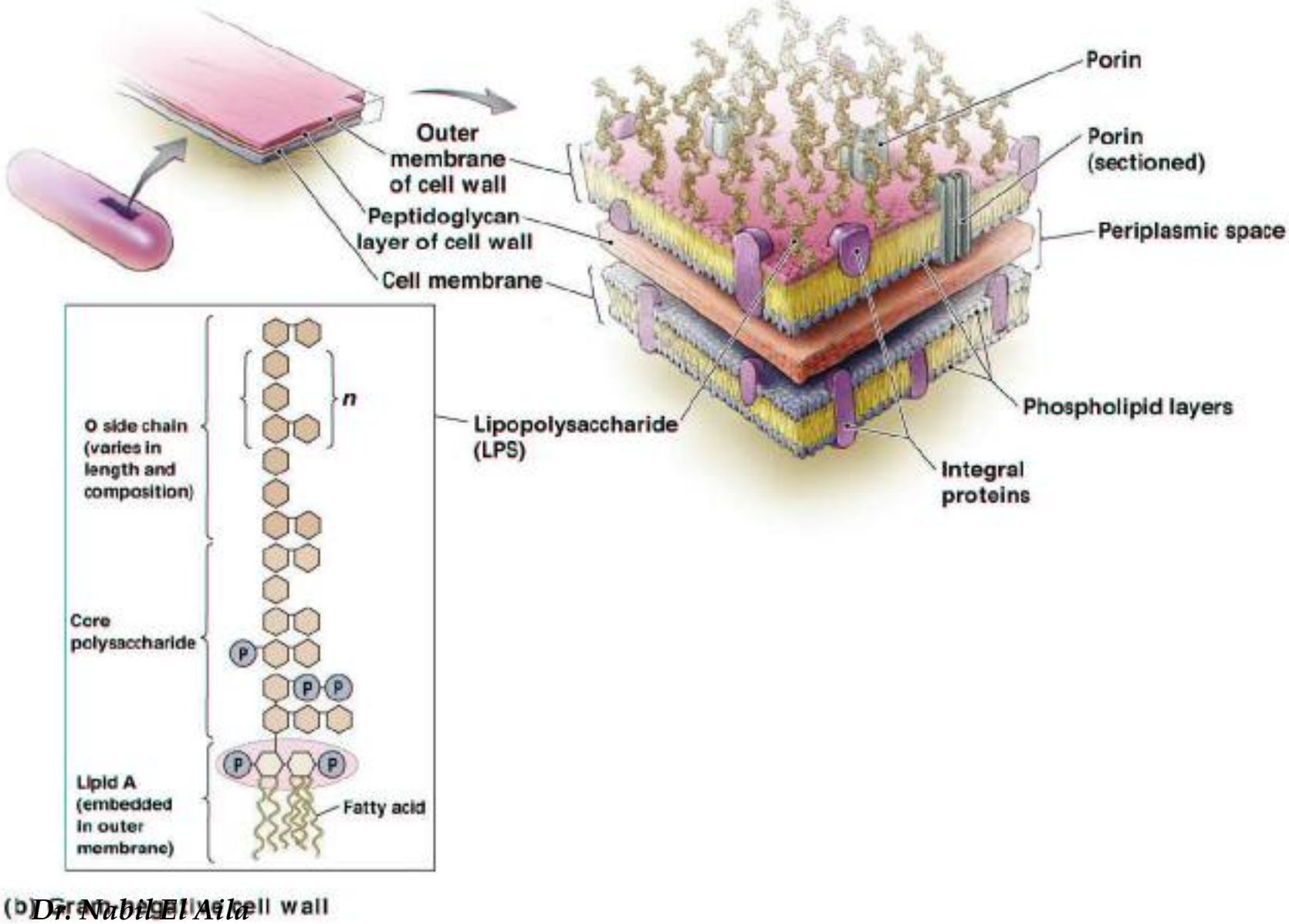
Structure	Chemical Composition	Function
Essential components		
Cell wall		
Peptidoglycan	Glycan (sugar) backbone with peptide side chains that are cross-linked	Gives rigid support, protects against osmotic pressure, is the site of action of penicillins and cephalosporins, and is degraded by lysozyme
Outer membrane of gram-negative bacteria	Lipid A	Toxic component of endotoxin
	Polysaccharide	Major surface antigen used frequently in laboratory diagnosis
Surface fibers of gram-positive bacteria	Teichoic acid	Major surface antigen but rarely used in laboratory diagnosis
Plasma membrane	Lipoprotein bilayer without sterols	Site of oxidative and transport enzymes
Ribosome	RNA and protein in 50S and 30S subunits	Protein synthesis; site of action of aminoglycosides, erythromycin, tetracyclines, and chloramphenicol
Nucleoid	DNA	Genetic material
Mesosome	Invagination of plasma membrane	Participates in cell division and secretion
Periplasm	Space between plasma membrane and outer membrane	Contains many hydrolytic enzymes, including β-lactamases
Nonessential components		
Capsule	Polysaccharide[1]	Protects against phagocytosis
Pilus or fimbria	Glycoprotein	Two types: (1) mediates attachment to cell surfaces; (2) sex pilus mediates attachment of two bacteria during conjugation
Flagellum	Protein	Motility
Spore	Keratinlike coat, dipicolinic acid	Provides resistance to dehydration, heat, and chemicals
Plasmid	DNA	Contains a variety of genes for antibiotic resistance and toxins
Granule	Glycogen, lipids, polyphosphates	Site of nutrients in cytoplasm
Glycocalyx	Polysaccharide	Mediates adherence to surfaces

Cell Wall

- The cell wall has several other important properties:
- (1) In Gram-negative bacteria, it contains endotoxin, a lipopolysaccharide
- (2) Its polysaccharides and proteins are antigens that are useful in laboratory identification.
- (3) Its porin proteins play a role in facilitating the passage of small, hydrophilic molecules into the cell.

Porin proteins in the outer membrane of gram-negative bacteria act as a channel to allow the entry of essential substances

Gram-negative cell wall

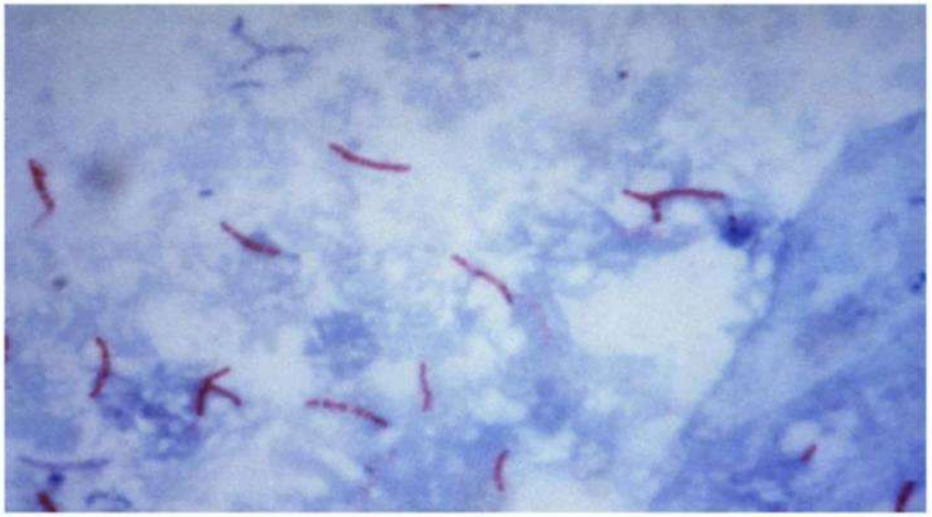


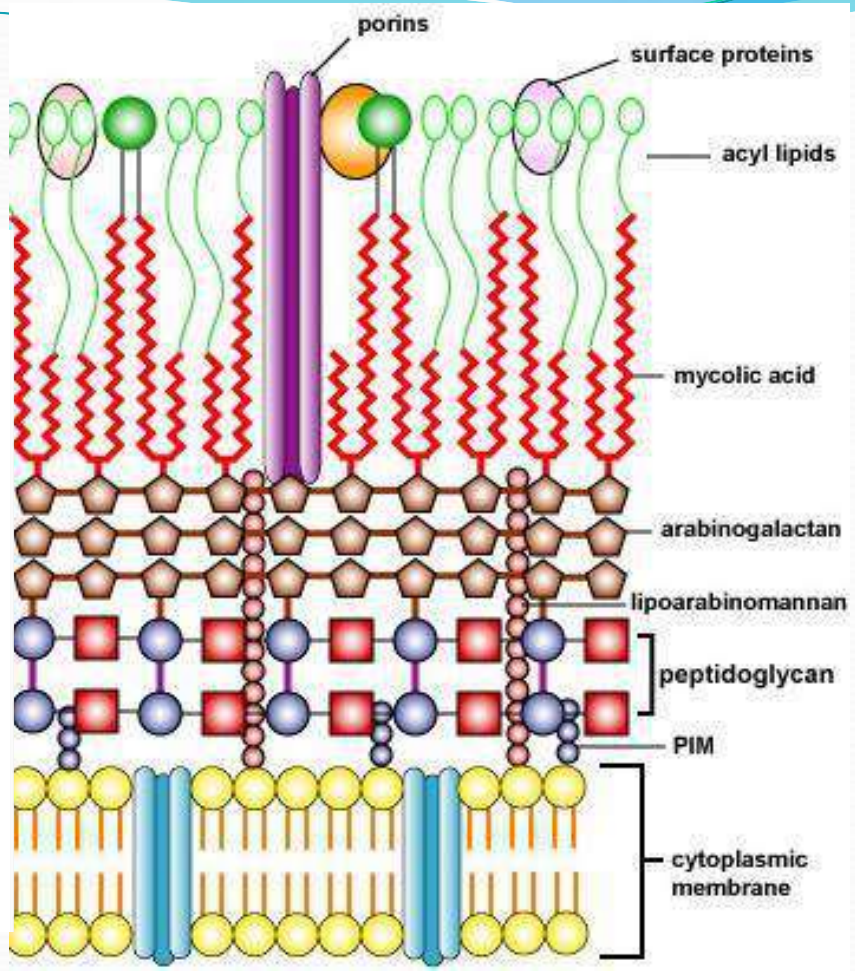
(b) Gram-negative cell wall

Cell Walls of Acid-Fast Bacteria

- Mycobacteria (e.g., Mycobacterium tuberculosis) have an unusual cell wall, resulting in their inability to be Gram-stained
- These bacteria are said to be acid-fast because they resist decolorization with acid–alcohol after being stained with carbolfuchsin.
- This property is related to the high concentration of lipids, called mycolic acids, in the cell wall of mycobacteria.
- Nocardia asteroides is weakly acid-fast.

Mycobacterium tuberculosis - Ziehl-Neelsen Staining



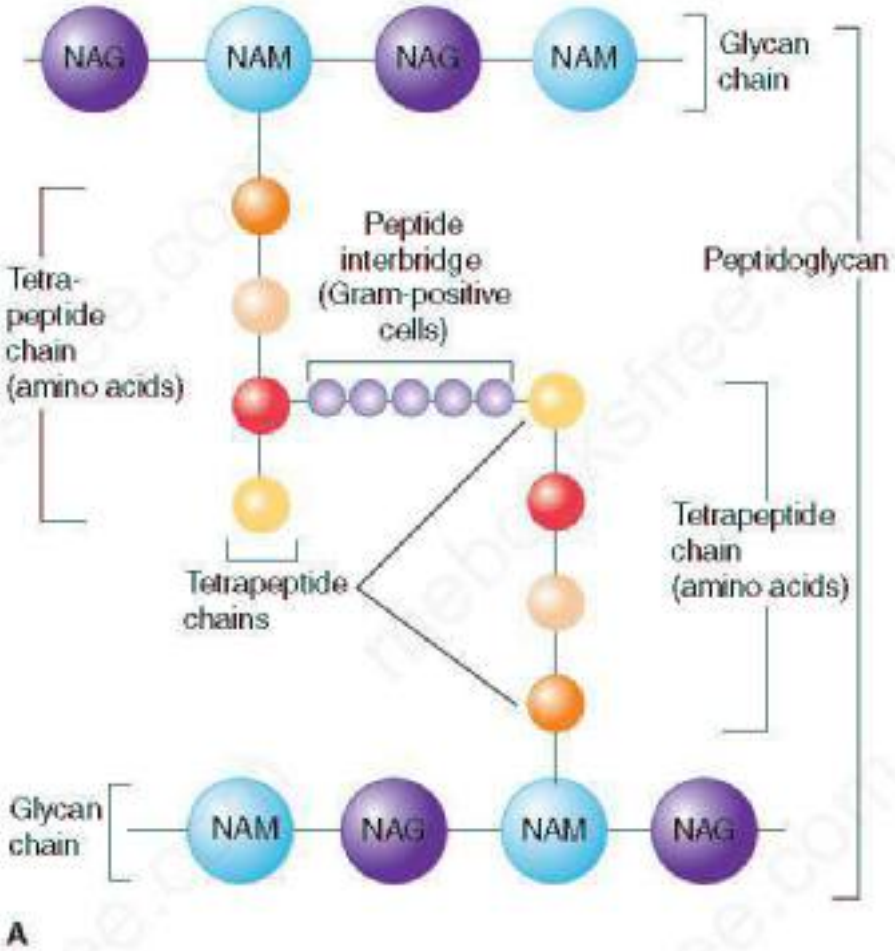


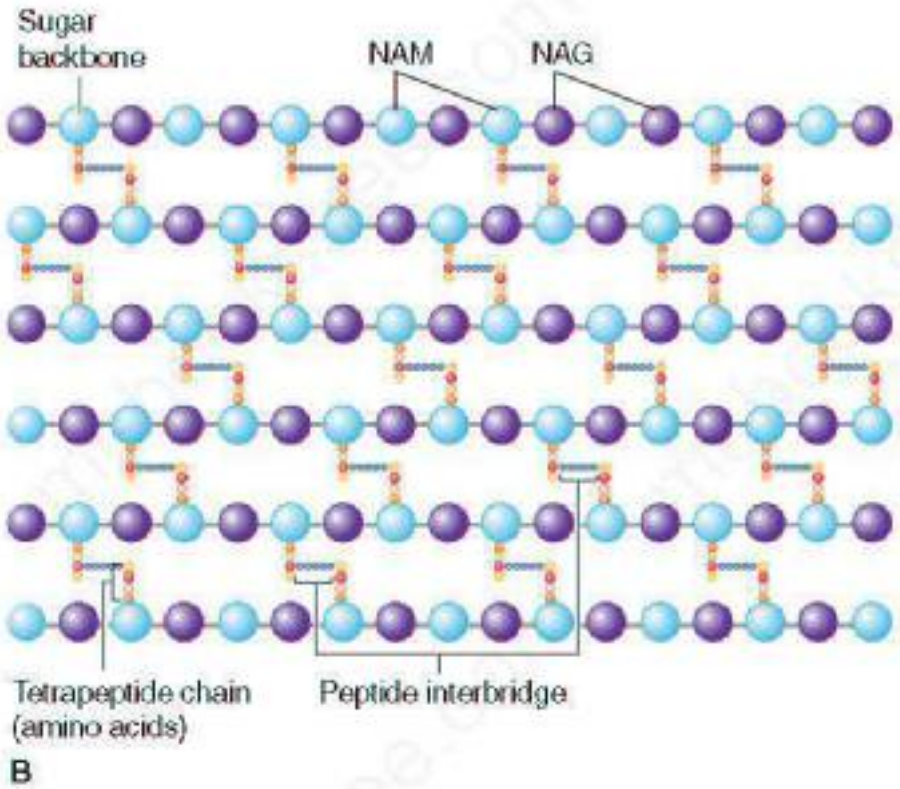
Structure of an Acid-Fast Cell Wall.

Peptidoglycan

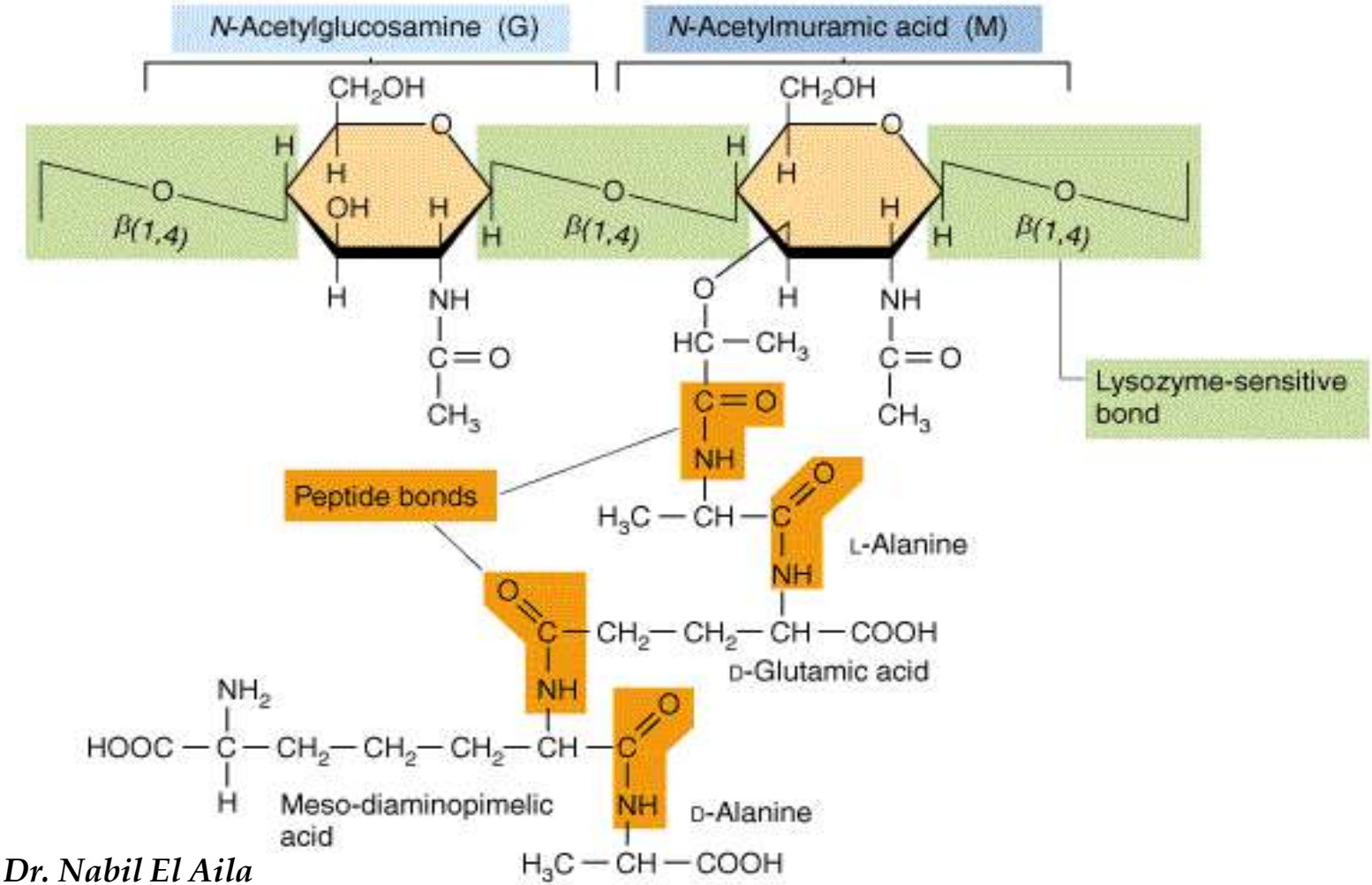
- Peptidoglycan is a complex, interwoven network that surrounds the entire cell and is composed of a single covalently linked macromolecule.
- It is found only in bacterial cell walls. It provides rigid support for the cell, is important in maintaining the characteristic shape of the cell, and allows the cell to withstand media of low osmotic pressure, such as water.
- The term peptidoglycan is derived from the peptides and the sugars (glycan) that make up the molecule. Synonyms for peptidoglycan are murein and mucopeptide.

- The carbohydrate backbone, which is composed of alternating N-acetylmuramic acid and N-acetylglucosamine molecules.
- Attached to each of the muramic acid molecules is a tetrapeptide consisting of both D- and L-amino acids, the precise composition of which differs from one bacterium to another.
- Two of these amino acids are : diaminopimelic acid, which is unique to bacterial cell walls, and d-alanine, which is involved in the cross-links between the tetrapeptides and in the action of penicillin.
- This tetrapeptide contains the rare d-isomers of amino acids, most proteins contain the l-isomer
- The other important component in this network is the peptide cross-link between the two tetrapeptides.
- Peptidoglycan is a good target for antibacterial drugs inhibit the synthesis of peptidoglycan by inhibiting the transpeptidase that makes the cross-links between the two adjacent tetrapeptides


NAG
NAM
NAG
NAM
Glycan chain
Peptidoglycan
Tetra-peptide chain (amino acids)
Peptide interbridge (Gram-positive cells)
Tetrapeptide chains
Tetrapeptide chain (amino acids)
Glycan chain
NAM
NAG
NAM
NAG
A


Sugar backbone
NAM
NAG
Tetrapeptide chain (amino acids)
Peptide interbridge
B

Structure of peptidoglycan



Lysozyme

- An enzyme present in human tears, mucus, and saliva, can cleave the peptidoglycan backbone by breaking its glycosyl bonds
- Lysozyme contributing to the natural resistance of the host to microbial infection.
- Lysozyme- treated bacteria may swell and rupture as a result of the entry of water into the cells, which have a high internal osmotic pressure.
- If the lysozyme-treated cells are in a solution with the same osmotic pressure as that of the bacterial interior, they will survive as spherical forms, called protoplasts, surrounded only by a cytoplasmic membrane.

Lipopolysaccharide

- The lipopolysaccharide (LPS) of the outer membrane of the cell wall of gram-negative bacteria is endotoxin
- It is responsible for many of the features of disease, such as fever and shock (especially hypotension), caused by these organisms
- It is called endotoxin because it is an integral part of the cell wall, in contrast to exotoxins, which are actively secreted from the bacteria

The LPS is composed of three distinct units

- (1) A phospholipid called lipid A, which is responsible for the toxic effects.
- (2) A core polysaccharide of five sugars linked through ketodeoxyoctulonate (KDO) to lipid A.
- (3) An outer polysaccharide consisting of up to 25 repeating units of three to five sugars.
- This outer polymer is the important somatic, or O, antigen of several gram negative bacteria that is used to identify certain organisms in the clinical laboratory.
- Some bacteria, notably members of the genus Neisseria, have an outer lipooligosaccharide (LOS) containing very few repeating units of sugars.

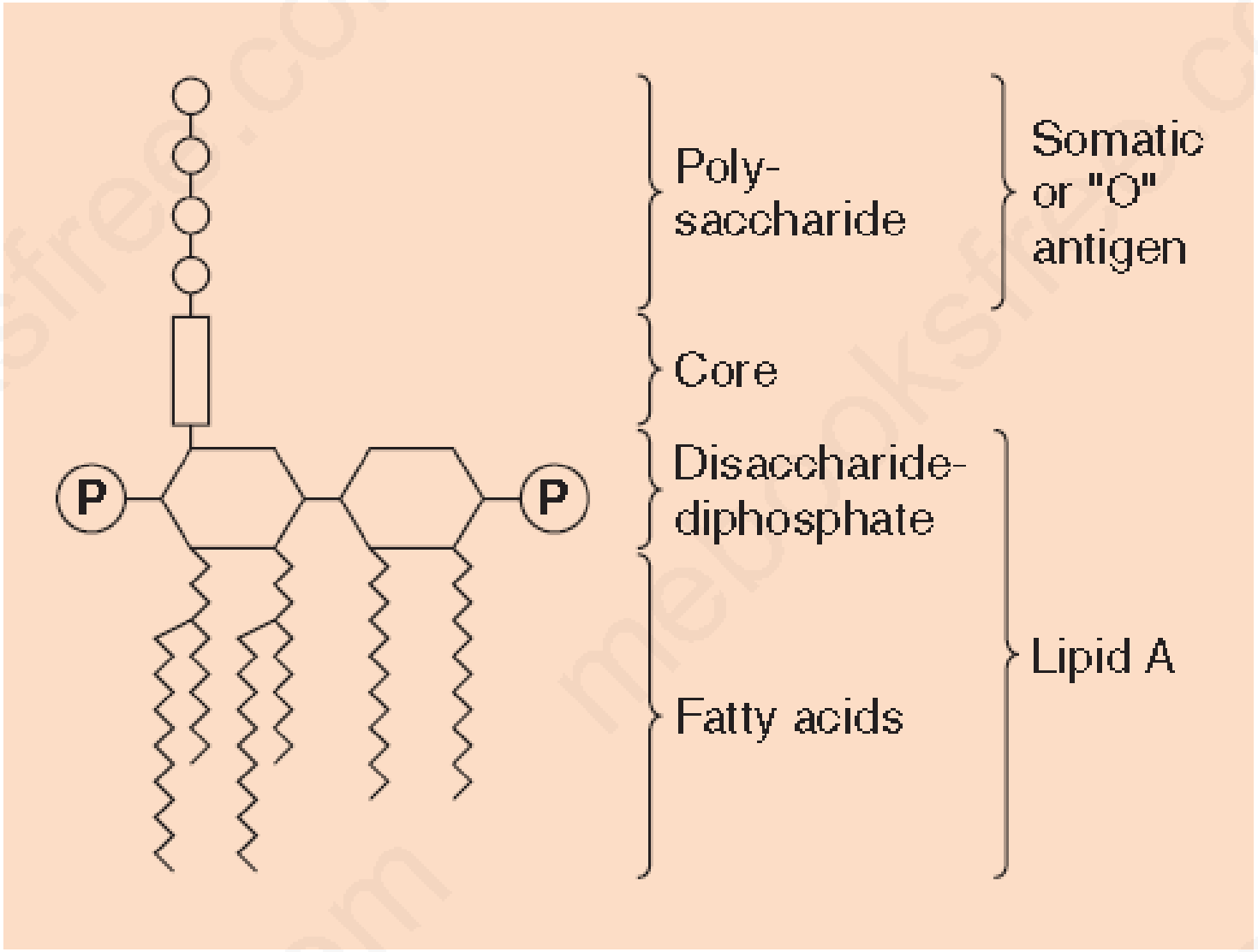
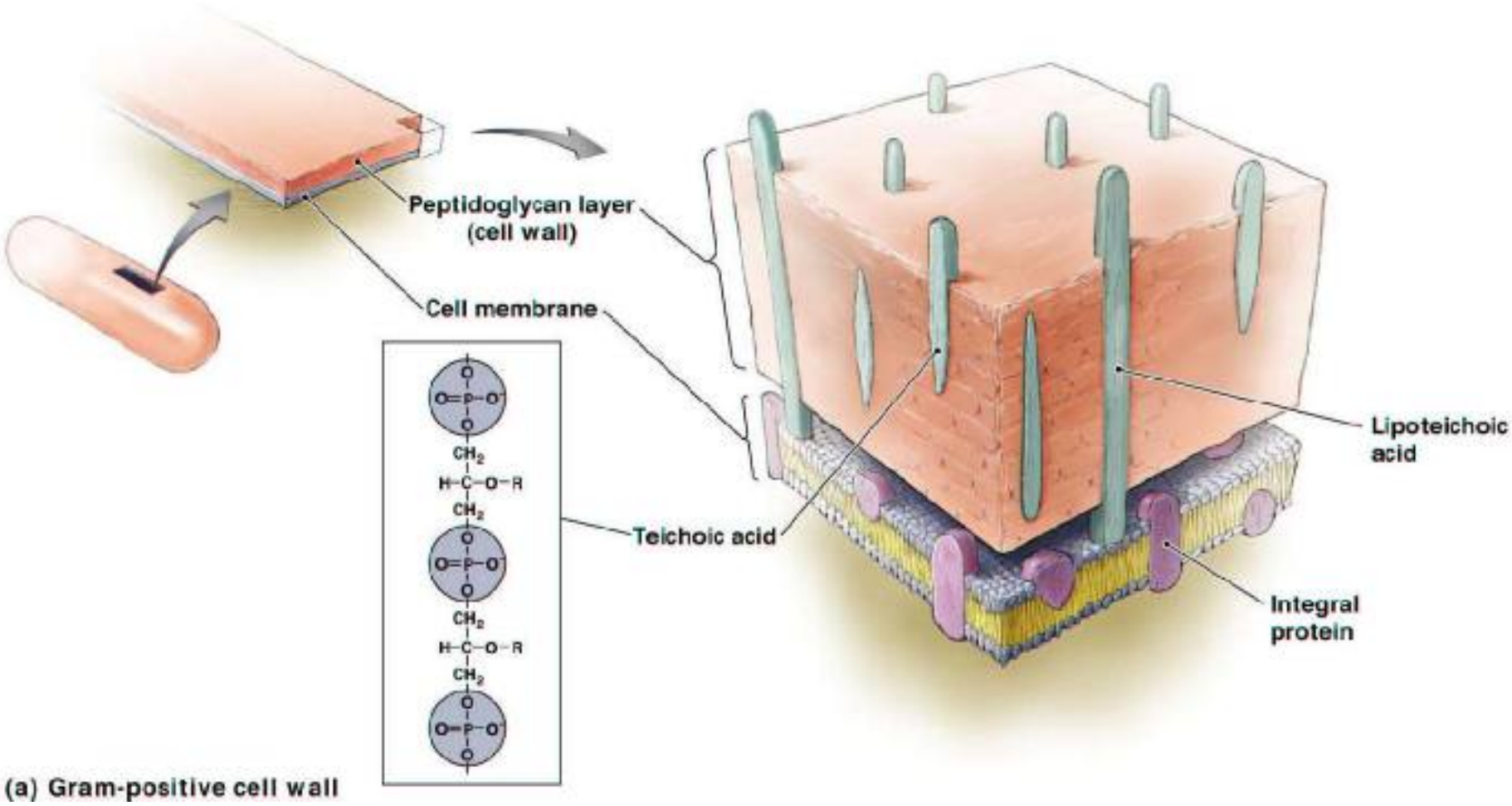


FIGURE 2–6 Endotoxin (lipopolysaccharide [LPS]) structure. Th
O-antigen polysaccharide is exposed on the exterior of the cell,

Teichoic Acid

- Teichoic acids are fibers located in the outer layer of the gram-positive cell wall and extend from it.
- They are composed of polymers of either glycerol phosphate or ribitol phosphate.
- Some polymers of glycerol teichoic acid penetrate the peptidoglycan layer and are covalently linked to the lipid in the cytoplasmic membrane, in which case the are called lipoteichoic acid
- The medical importance of teichoic acids lies in their ability to induce inflammation and septic shock when caused by certain gram-positive bacteria
- Teichoic acids also mediate the attachment of staphylococci to mucosal cells

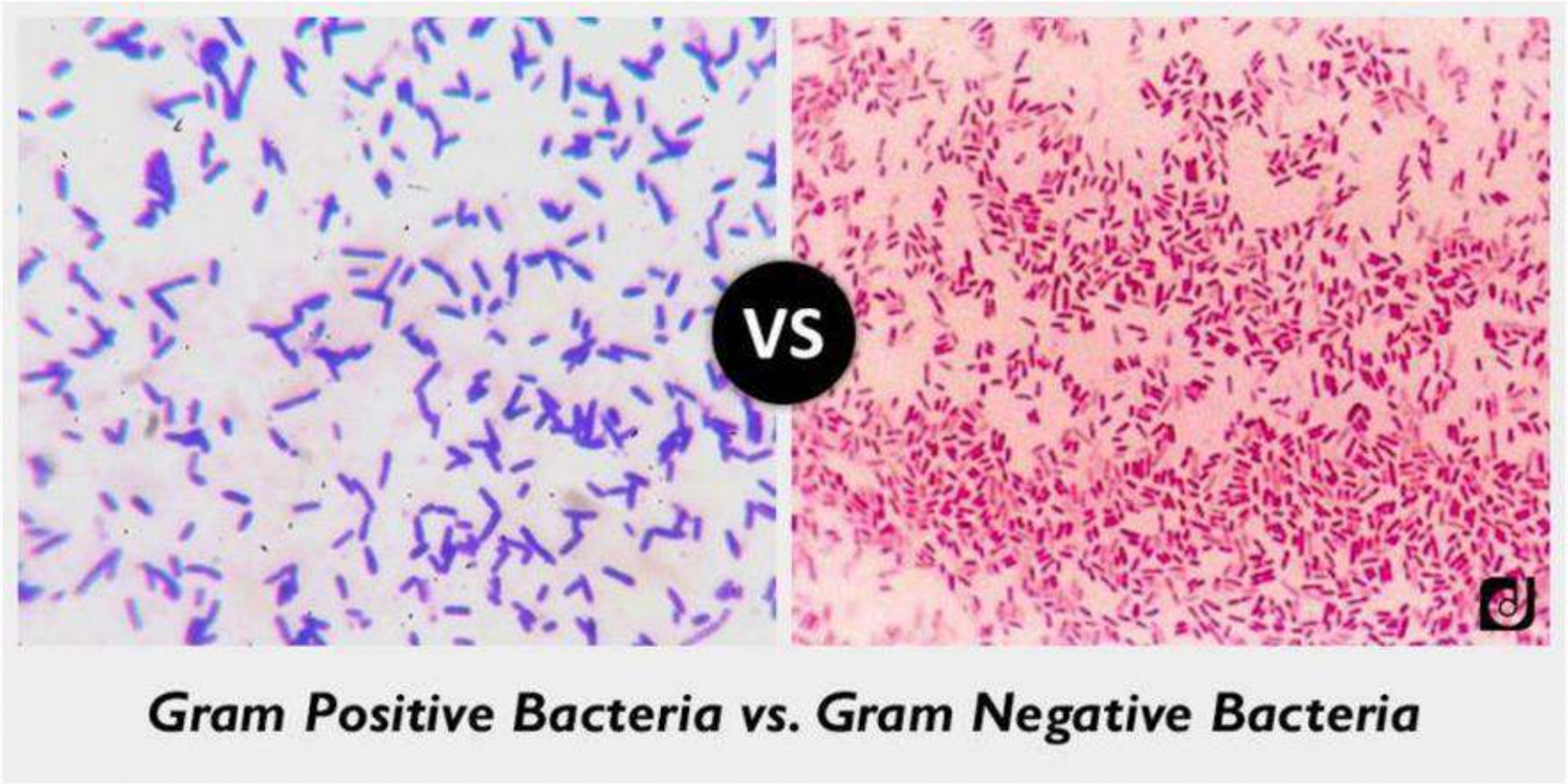
Bacterial Cell Walls



Gram stain

- It separates most bacteria into two groups:
- the gram-positive bacteria, which stain blue, and the gram negative bacteria, which stain red.

  The Gram stain involves the following four-step procedure:
- (1) The crystal violet dye stains all cells blue/purple.
- (2) The iodine solution (a mordant) is added to form a crystal violet–iodine complex; all cells continue to appear blue.
- (3) The organic solvent, such as acetone or ethanol, extracts the blue dye complex from the lipid-rich, thin-walled gram-negative bacteria to a greater degree than from the lipid-poor, thick-walled gram-positive bacteria.
- The gram-negative organisms appear colorless; the gram-positive bacteria remain blue.
- (4) The red dye safranin stains the decolorized gram negative cells red/pink; the gram-positive bacteria remain blue.



Gram Positive Bacteria vs. Gram Negative Bacteria

Cytoplasmic Membrane

- It is composed of a phospholipid bilayer
- Eukaryotic membranes contain sterols, whereas prokaryotes generally do not. The only prokaryotes that have sterols in their membranes are members of the genus Mycoplasma

The membrane has four important functions:

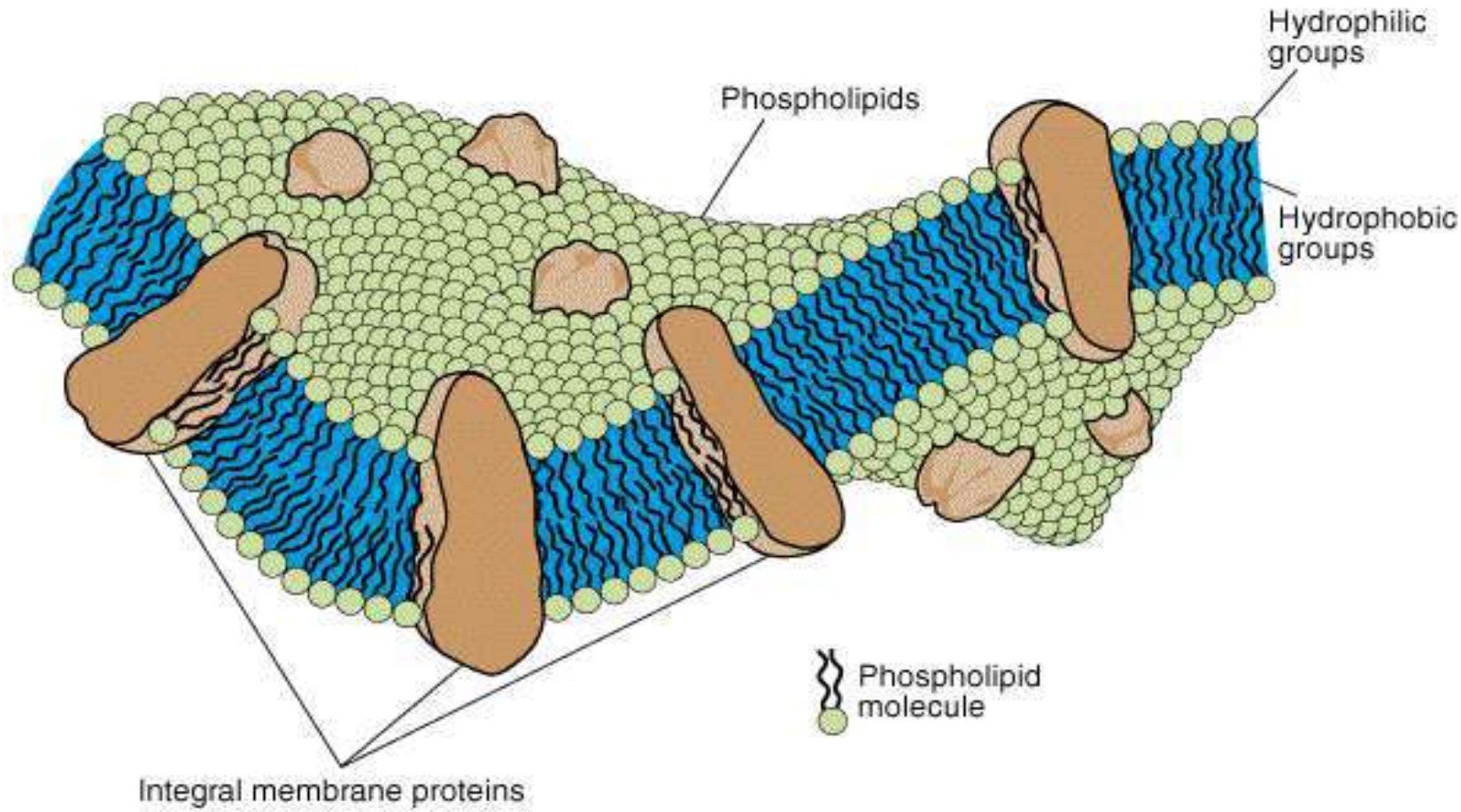
(1) active transport of molecules into the cell,

(2) energy generation by oxidative phosphorylation,

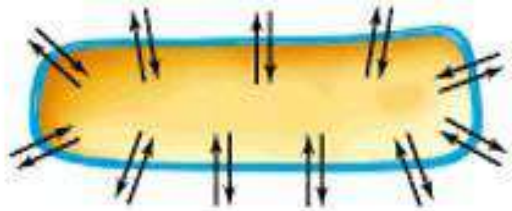
(3) synthesis of precursors of the cell wall,

(4) secretion of enzymes and toxins.

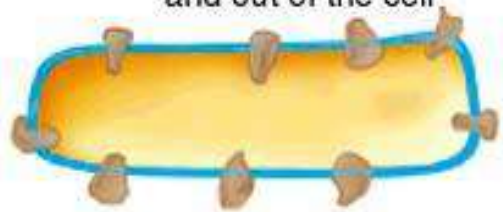
The cytoplasmic membrane



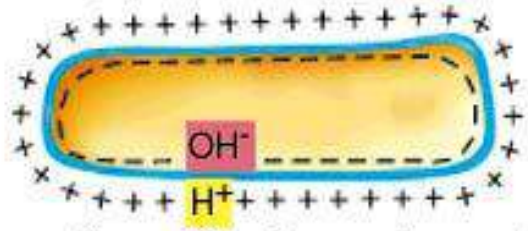
Functions of the cytoplasmic membrane(1)



Permeability Barrier — Prevents leakage and functions as a gateway for transport of nutrients into and out of the cell



Protein Anchor — Site of many proteins involved in transport, bioenergetics, and chemotaxis



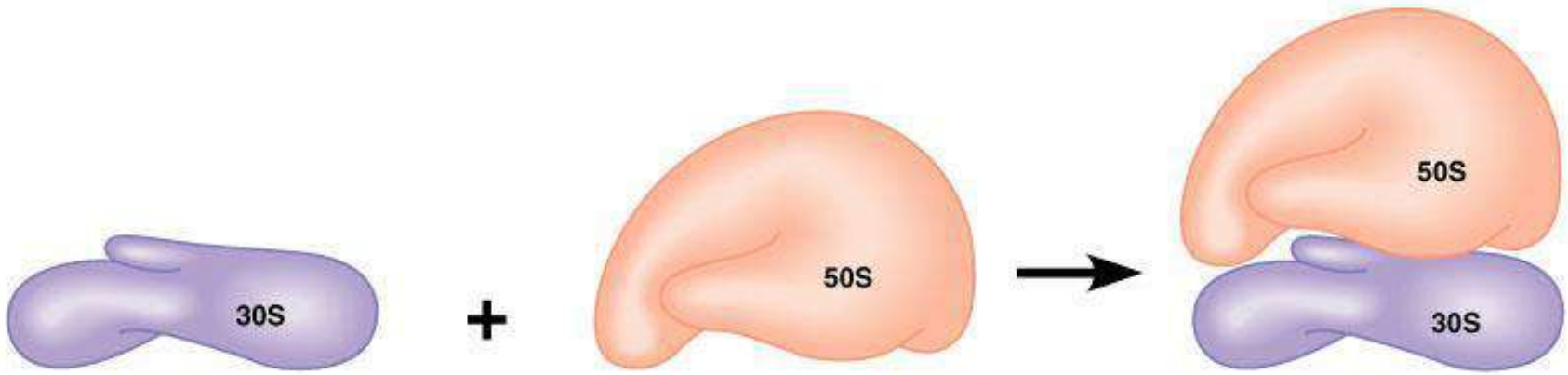
Energy Conservation — Site of generation and use of the proton motive force

Cytoplasm

- The cytoplasm has two distinct areas when seen in the electron microscope:
- (1) An amorphous matrix that contains ribosomes, nutrient granules, metabolites, and plasmids.
- (2) An inner, nucleoid region composed of DNA.

Ribosomes

- Bacterial ribosomes are the site of protein synthesis as in eukaryotic cells, but they differ from eukaryotic ribosomes in size and chemical composition.
- Bacterial ribosomes are 70S in size, with 50S and 30S subunits, whereas eukaryotic ribosomes are 80S in size, with 60S and 40S subunits.
- The differences in both the ribosomal RNAs and proteins constitute the basis of the selective action of several antibiotics that inhibit bacterial, but not human, protein synthesis



(a) Small subunit **(b) Large subunit** **(c) Complete 70S ribosome**

Granules

- The cytoplasm contains several different types of granules that serve as storage areas for nutrients and stain characteristically with certain dyes.
- For example, volutin is a reserve of high energy stored in the form of polymerized metaphosphate.
- It appears as a "metachromatic" granule since it stains red with methylene blue dye instead of blue as one would expect.
- Metachromatic granules are a characteristic feature of Corynebacterium diphtheriae, the cause of diphtheria.

Nucleoid

- **The nucleoid** is the area of the cytoplasm in which DNA is located.
- The DNA of prokaryotes is a single, circular molecule that has a molecular weight (MW) of approximately 2×10^9 and contains about 2000 genes.(By contrast, human DNA has approximately 100,000 genes.)
- The nucleoid contains no nuclear membrane, no nucleolus, no mitotic spindle, and no histones
- One major difference between bacterial DNA and eukaryotic DNA is that bacterial DNA has no introns, whereas eukaryotic DNA does.

Plasmids

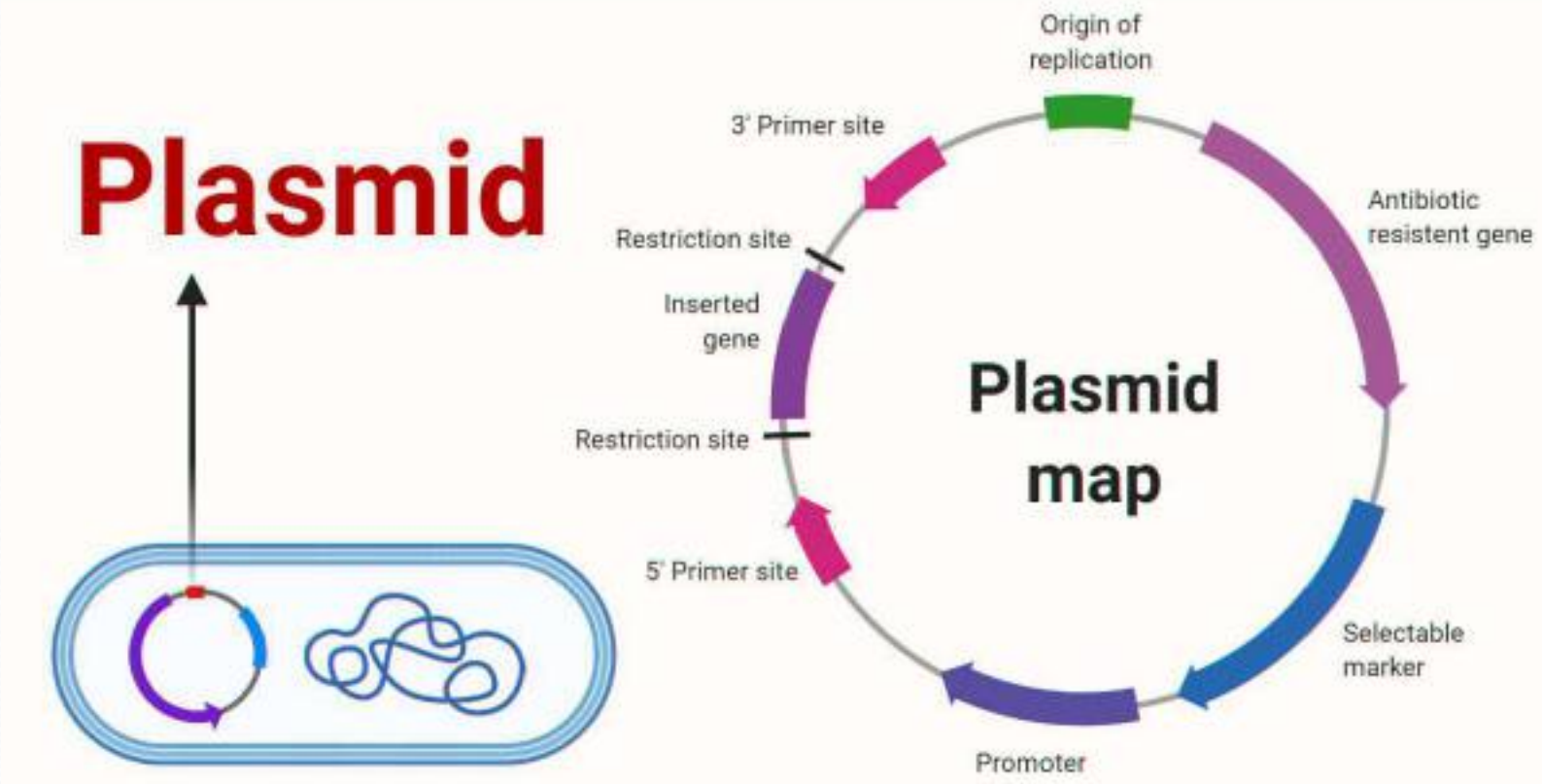
- Plasmids are extrachromosomal, double-stranded, circular DNA molecules that are capable of replicating independently of the bacterial chromosome.
- Plasmids occur in both gram-positive and gram-negative bacteria

(1) Transmissible plasmids:

- Can be transferred from cell to cell by conjugation
- They are large (MW 40–100 million), since they contain about a dozen genes responsible for the synthesis of the sex pilus and for the enzymes required for transfer.
- They are usually present in a few (1–3) copies per cell.

- Non transmissible plasmids are small (MW 3–20 million), since they do not contain the transfer genes; they are frequently present in many (10–60) copies per cell.
- Plasmids carry the genes for the following functions;
- (1) Antibiotic resistance, which is mediated by a variety of enzymes, such as the beta-lactamase of S. aureus, Escherichia coli, and Klebsiella pneumoniae.
- (2) Exotoxins, such as the enterotoxins of E. coli, anthrax toxin of Bacillus anthracis, exfoliative toxin of S. aureus and tetanus toxin of Clostridium tetani.
- (3) Pili (fimbriae), which mediate the adherence of bacteria to epithelial cells.

- (4) Resistance to heavy metals, such as mercury, the active component of some antiseptics (e.g., merthiolate and mercurochrome), and silver, which is mediated by a reductase enzyme.
- (5) Resistance to ultraviolet light, which is mediated by DNA repair enzymes.


Plasmid
Origin of replication
3' Primer site
Antibiotic resistent gene
Restriction site
Inserted gene
Plasmid map
Restriction site
5' Primer site
Selectable marker
Promoter

- **Other plasmid-encoded products of interest are as follows:**
- (1) Bacteriocins are toxic proteins produced by certain bacteria that are lethal for other bacteria.

Two common mechanisms of action of bacteriocins are

- (i) degradation of bacterial cell membranes by producing pores in the membrane and
- (ii) degradation of bacterial DNA by DNAse.

colicins made by E. coli and pyocins made by Pseudomonas aeruginosa.

- (2) Nitrogen fixation enzymes in Rhizobium in the root nodules of legumes.
- (3) Tumors caused by Agrobacterium in plants.
- (4) Several antibiotics produced by Streptomyces.
- (5) A variety of degradative enzymes that are produced by Pseudomonas

Transposons

- Transposons are pieces of DNA that move readily from one site to another either within or between the DNAs of bacteria, plasmids, and bacteriophages.

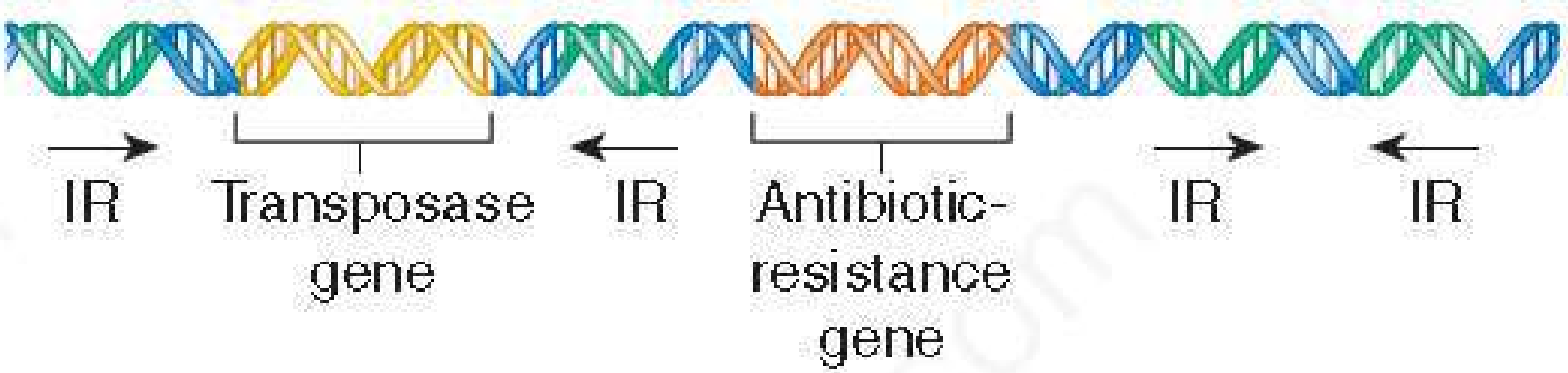
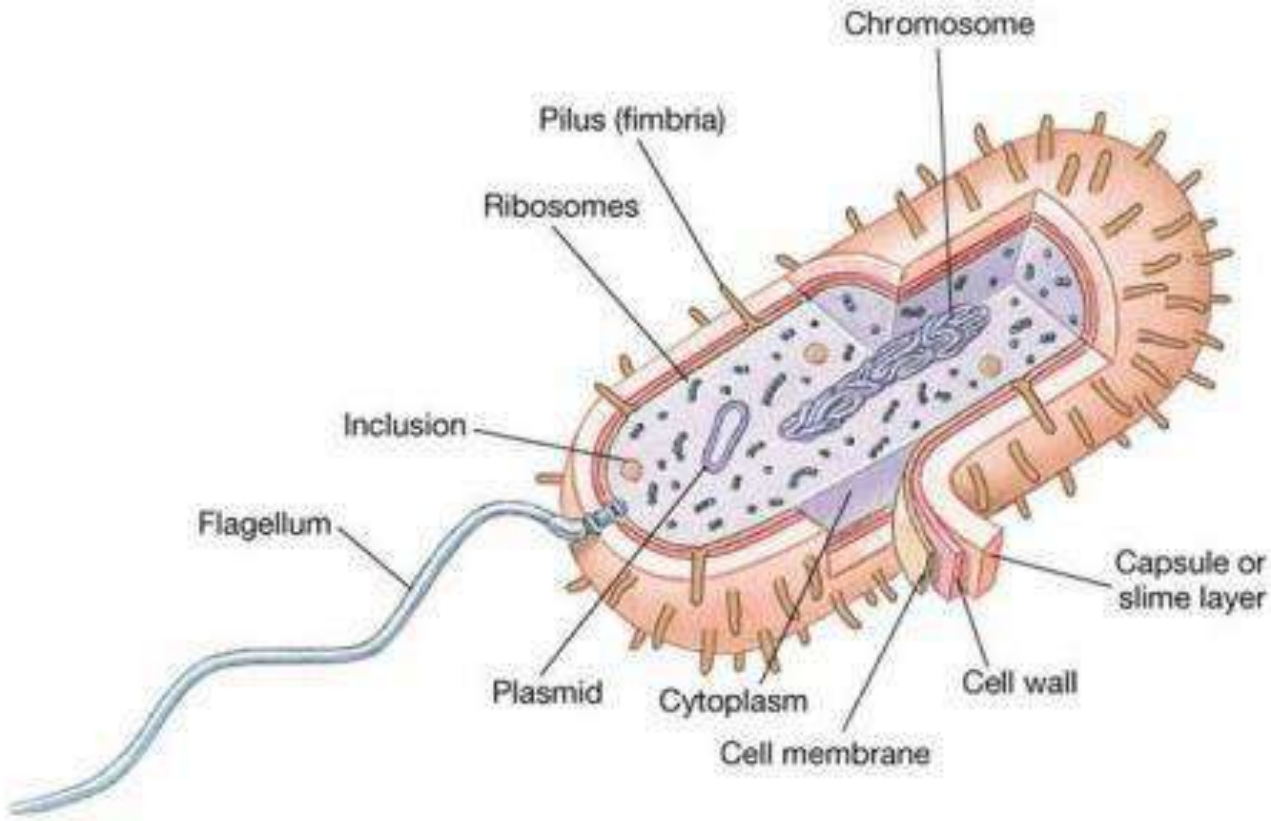


FIGURE 2–7 Transposon genes. This transposon is carrying a drug-resistance gene. IR, inverted repeat. (Reproduced with permission


Chromosome
Pilus (fimbria)
Ribosomes
Inclusion
Flagellum
Capsule or
slime layer
Plasmid
Cytoplasm
Cell wall
Cell membrane

Structures Outside the Cell Wall

Capsule:

- The capsule is a gelatinous layer covering the entire bacterium.
- It is composed of polysaccharide, except in the anthrax bacillus, which has a capsule of polymerized d-glutamic acid.
- **The capsule is important for four reasons:**
- It is a determinant of virulence of many bacteria since it limits the ability of phagocytes to engulf the bacteria.
- Specific identification of an organism can be made by using antiserum against the capsular polysaccharide.
- Capsular polysaccharides are used as the antigens in certain vaccines because they are capable of eliciting protective antibodies.
- The capsule may play a role in the adherence of bacteria to human tissues,

Flagella

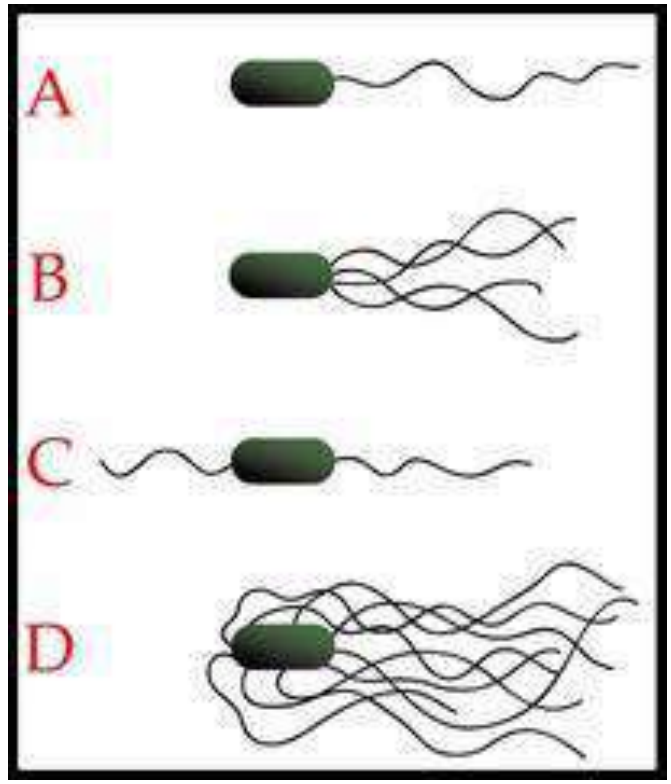
- Flagella are long, whiplike appendages that move the bacteria toward nutrients and other attractants, a process called chemotaxis.
- The long filament, which acts as a propeller, is composed of many subunits of a single protein, flagellin, arranged in several intertwined chains.
- The energy for movement, the proton motive force, is provided by adenosine triphosphate (ATP), derived from the passage of ions across the membrane.

Flagella are medically important for two reasons:

(1) Some species of motile bacteria (e.g., E. coli and Proteus species) are common causes of urinary tract infections.

- Flagella may play a role in pathogenesis by propelling the bacteria up the urethra into the bladder.

2) Some species of bacteria (e.g., Salmonella species) are identified in the clinical laboratory by the use of specific antibodies against flagellar proteins.



A. Monotrichous polar
B. Lophotrichous polar
C. Amphitrichous polar
D. Petrichous

Pili (Fimbriae)

- Pili are hairlike filaments that extend from the cell surface.
- Composed of subunits of pilin, a protein arranged in helical strands.
- They are found mainly on gram-negative organisms.

Pili have two important roles:

- (1) They mediate the attachment of bacteria to specific receptors on the human cell surface, which is a necessary step in the initiation of infection for some organisms.
- Mutants of Neisseria gonorrhoeae that do not form pili are nonpathogens.
- (2) A specialized kind of pilus, the sex pilus, forms the attachment between the male (donor) and the female (recipient) bacteria during conjugation

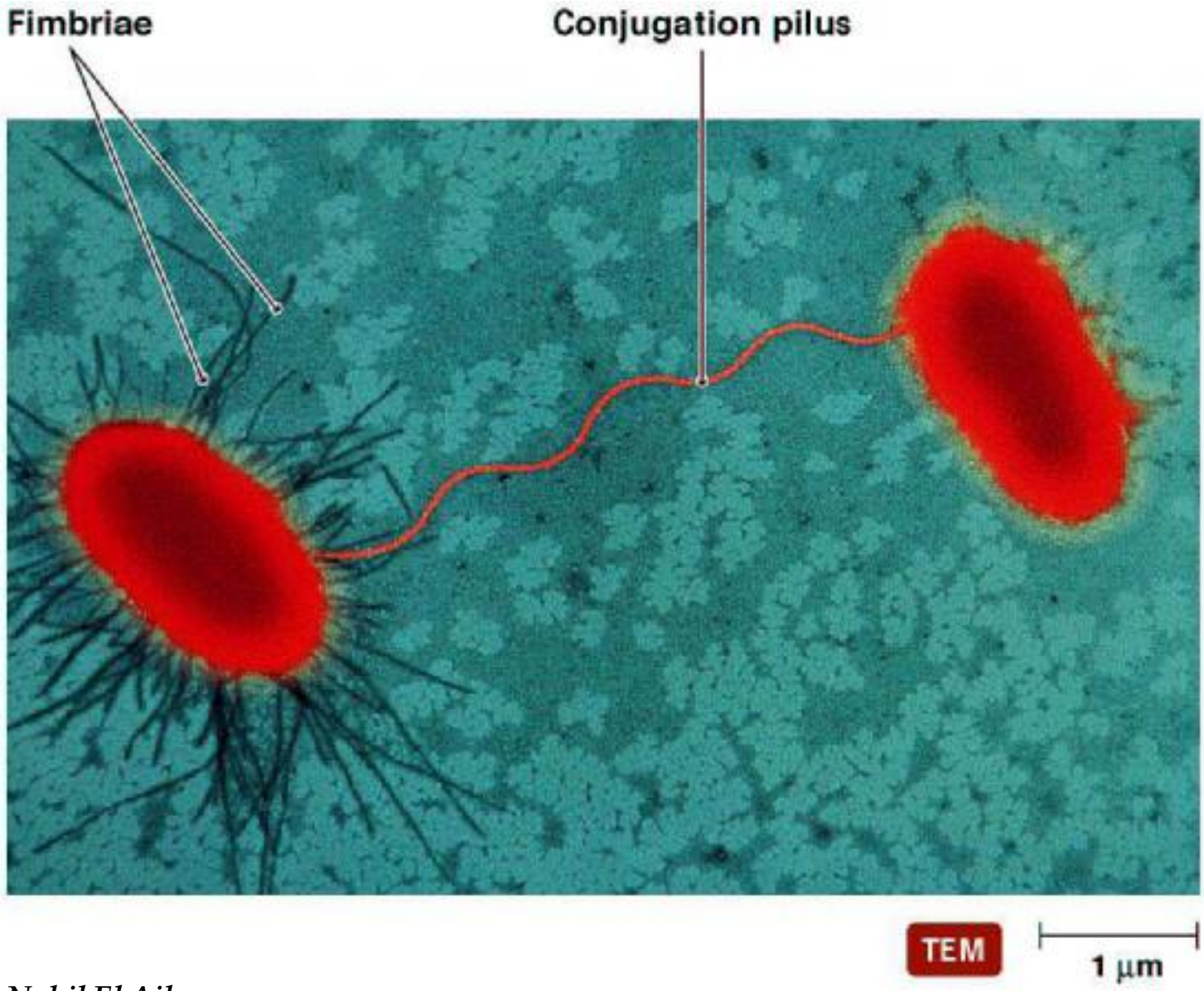


Figure 3.10

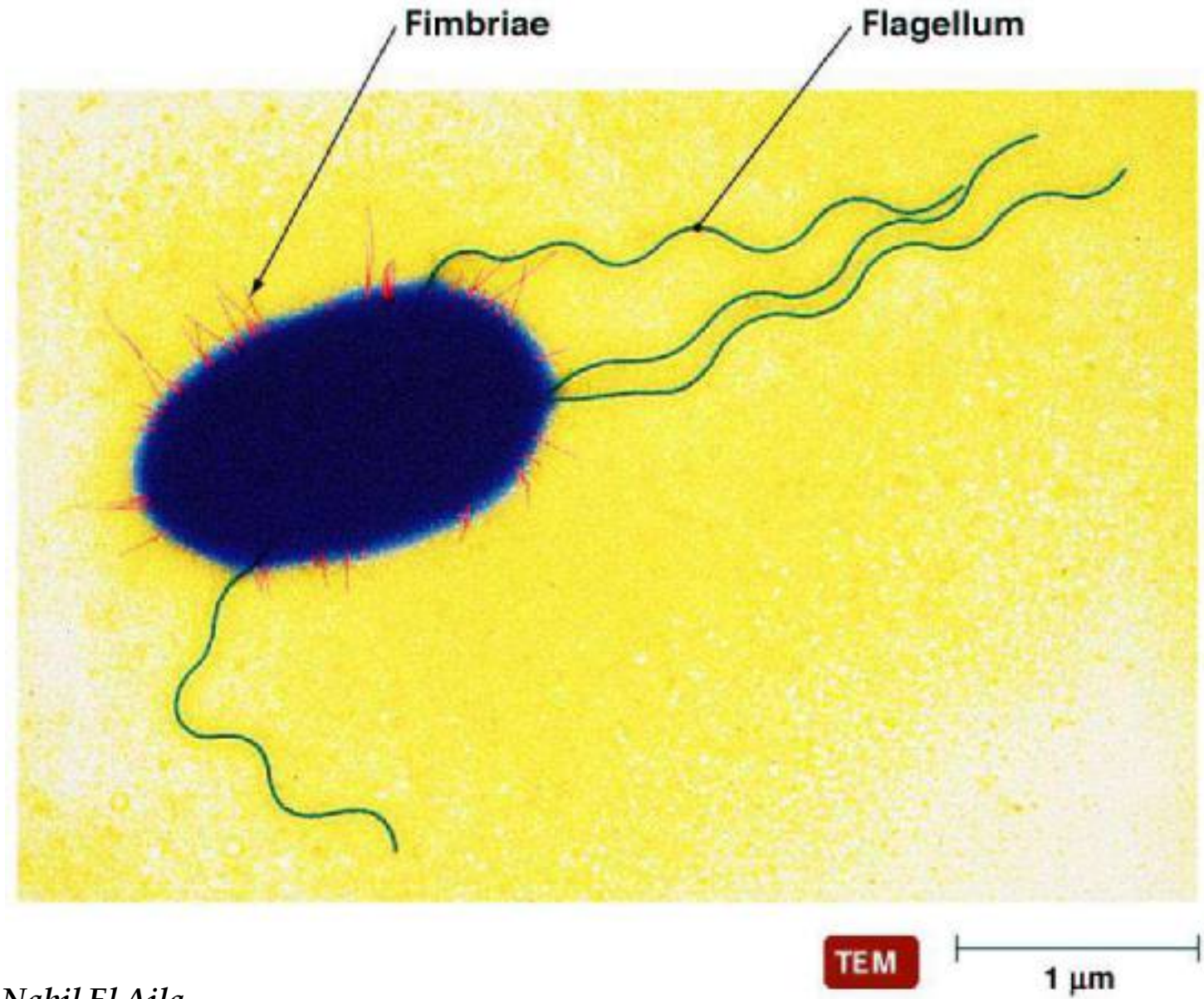


Figure 3.9

Glycocalyx (Slime Layer)

- The glycocalyx is a polysaccharide coating that is secreted by many bacteria.
- It covers surfaces like a film and allows the bacteria to adhere firmly to various structures (e.g., skin, heart valves, prosthetic joints, and catheters).
- The glycocalyxm is an important component of biofilms .
- It is the glycocalyx-producing strains of P. aeruginosa that cause respiratory tract infections in cystic fibrosis patients,
- It is the glycocalyx-producing strains of Staphylococcus epidermidis and viridans streptococci that cause endocarditis.
- The glycocalyx also mediates adherence of certain bacteria, such as Streptococcus mutans, to the surface of teeth. This plays an important role in the formation of plaque, the precursor of dental caries.

Bacterial Spores

- These highly resistant structures are formed in response to adverse conditions by two genera of medically important gram-positive rods:
- the genus Bacillus, which includes the agent of anthrax, and the genus Clostridium, which includes the agents of tetanus and botulism.
- Spore formation (sporulation) occurs when nutrients, such as sources of carbon and nitrogen, are depleted inside the cell
- It contains bacterial DNA, a small amount of cytoplasm, cell membrane, peptidoglycan, very little water, and most importantly, a thick, keratinlike coat that is responsible for the remarkable resistance of the spore to heat, dehydration, radiation, and chemicals.

- This resistance may be mediated by dipicolinic acid, a calcium ion chelator found only in spores.
- The medical importance of spores lies in their extraordinary resistance to heat and chemicals.
- As a result of their resistance to heat, sterilization cannot be achieved by
- boiling. Steam heating under pressure (autoclaving) at 121°C, for at least 15 minutes, is required to ensure the sterility of products for medical use.

Bacterial Spores Structure, Importance and examples

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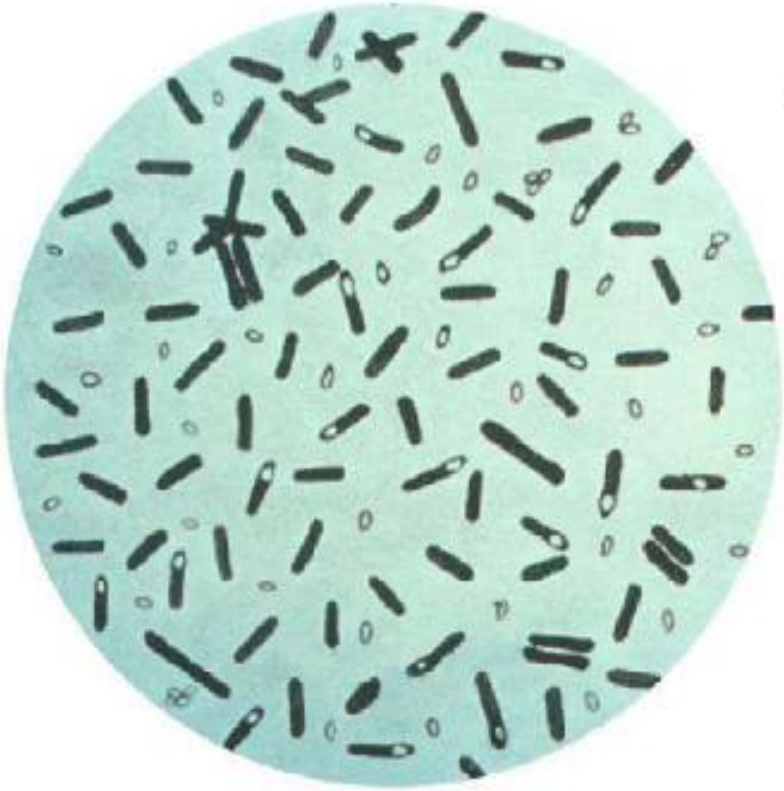
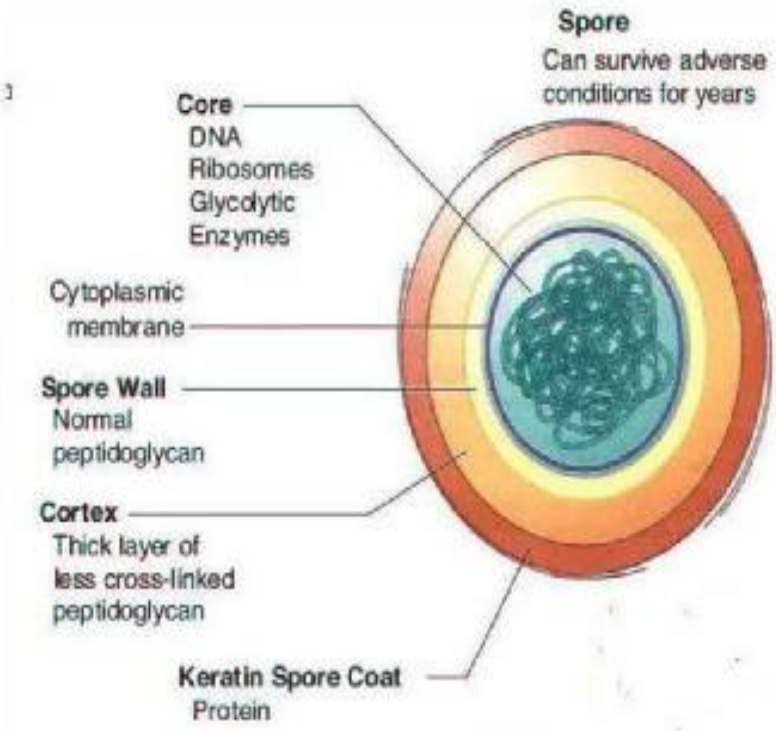




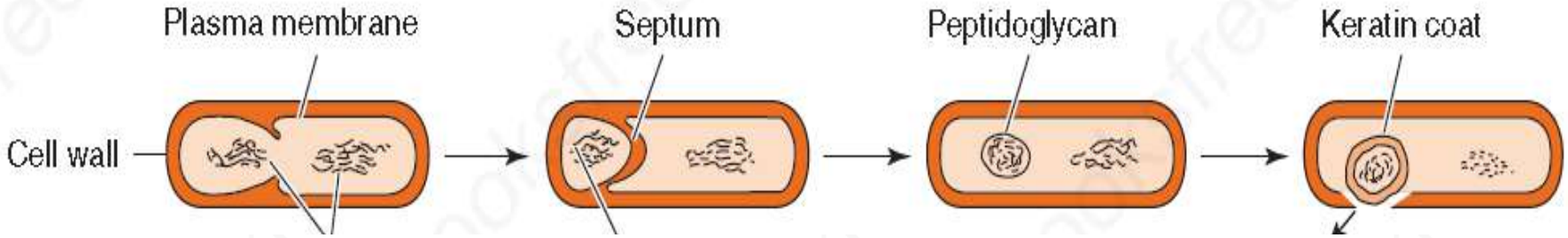
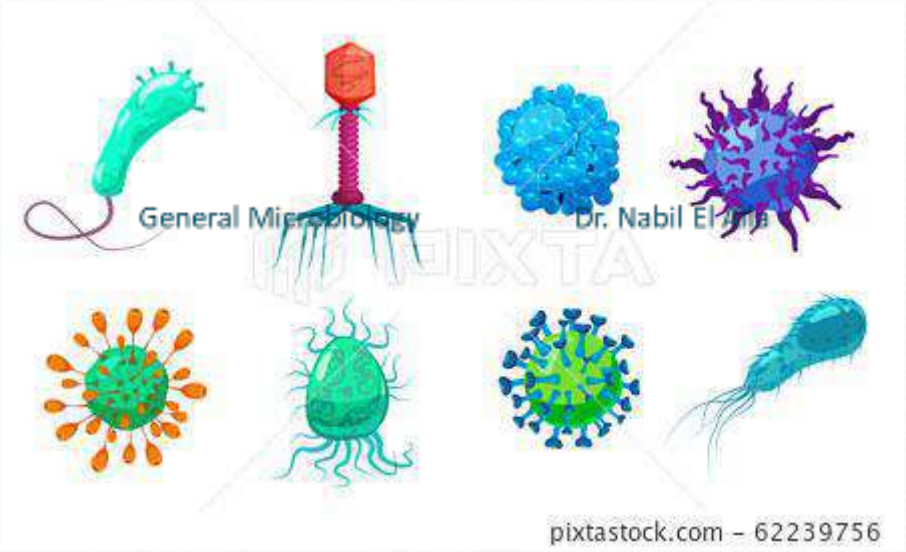


TABLE 2–4 Important Features of Spores and Their Medical Implications

Important Features of Spores	Medical Implications
Highly resistant to heating; spores are not killed by boiling (100°C), but are killed at 121°C.	Medical supplies must be heated to 121°C for at least 15 minutes to be sterilized.
Highly resistant to many chemicals, including most disinfectants, due to the thick, keratinlike coat of the spore.	Only solutions designated as sporicidal will kill spores.
They can survive for many years, especially in the soil.	Wounds contaminated with soil can be infected with spores and cause diseases such as tetanus (*C. tetani*) and gas gangrene (*C. perfringens*).
They exhibit no measurable metabolic activity.	Antibiotics are ineffective against spores because antibiotics act by inhibiting certain metabolic pathways of bacteria. Also, spore coat is impermeable to antibiotics.
Spores form when nutrients are insufficient but then germinate to form bacteria when nutrients become available.	Spores are not often found at the site of infections because nutrients are not limiting. Bacteria rather than spores are usually seen in Gram-stained smears.
Spores are produced by members of only two genera of bacteria of medical importance, *Bacillus* and *Clostridium*, both of which are gram-positive rods.	Infections transmitted by spores are caused by species of either *Bacillus or Clostridium*.

End of Lecture



TORTORA • FUNKE • CASE

Microbiology

AN INTRODUCTION

EIGHTH EDITION

Chapter 3

Microbial Growth

PowerPoint® Lecture Slide Presentation prepared by Christine L. Case

Microbial Growth

- Microbial growth = increase in number of cells, not cell size

The Requirements for Growth: Physical Requirements

- Temperature
  - Minimum growth temperature
  - Optimum growth temperature
  - Maximum growth temperature

Temperature

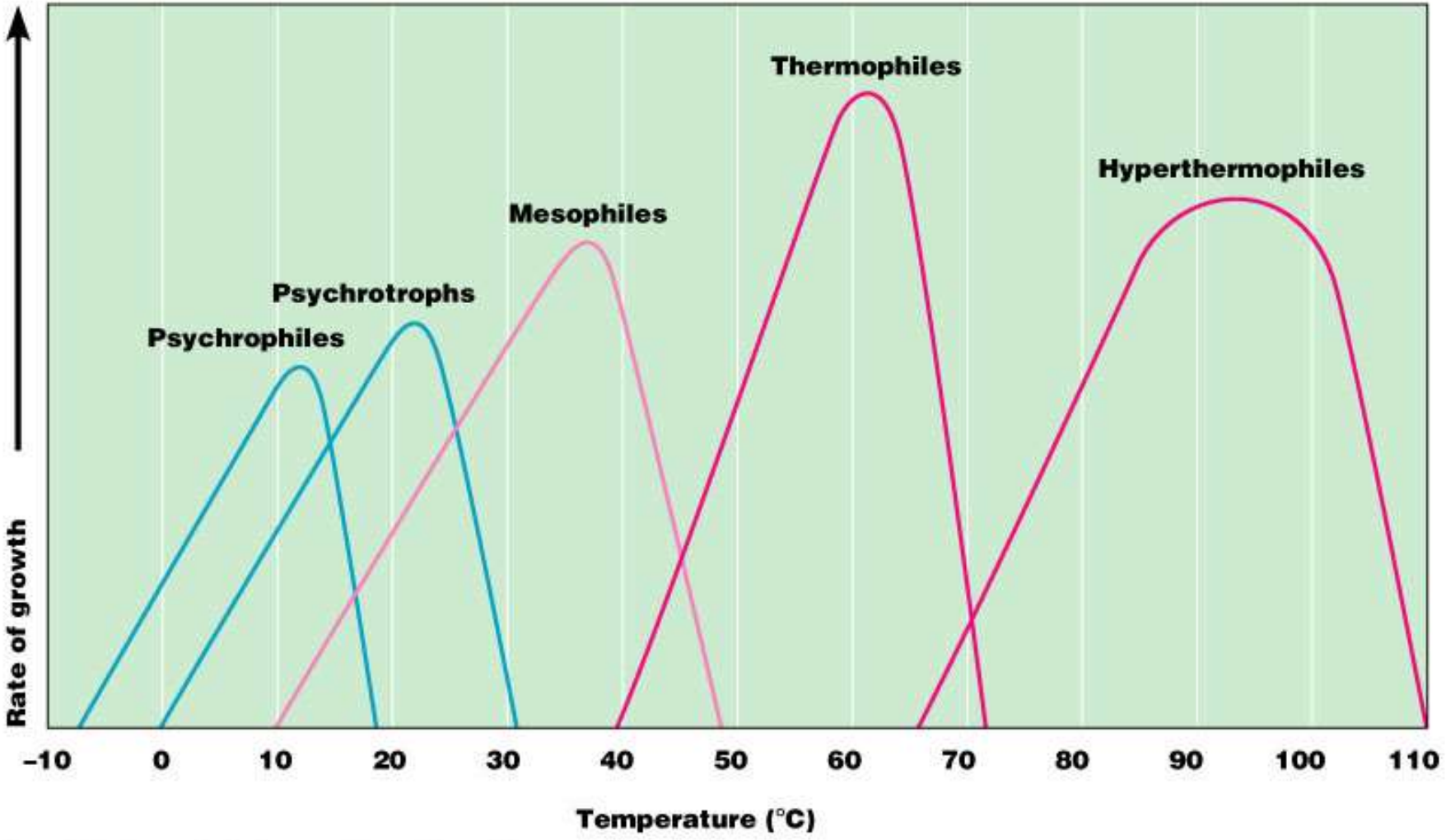


Figure 6.1

Psychrotrophs

- Grow between 0°C and 20-30°C
- Cause food spoilage

Psychrotrophs

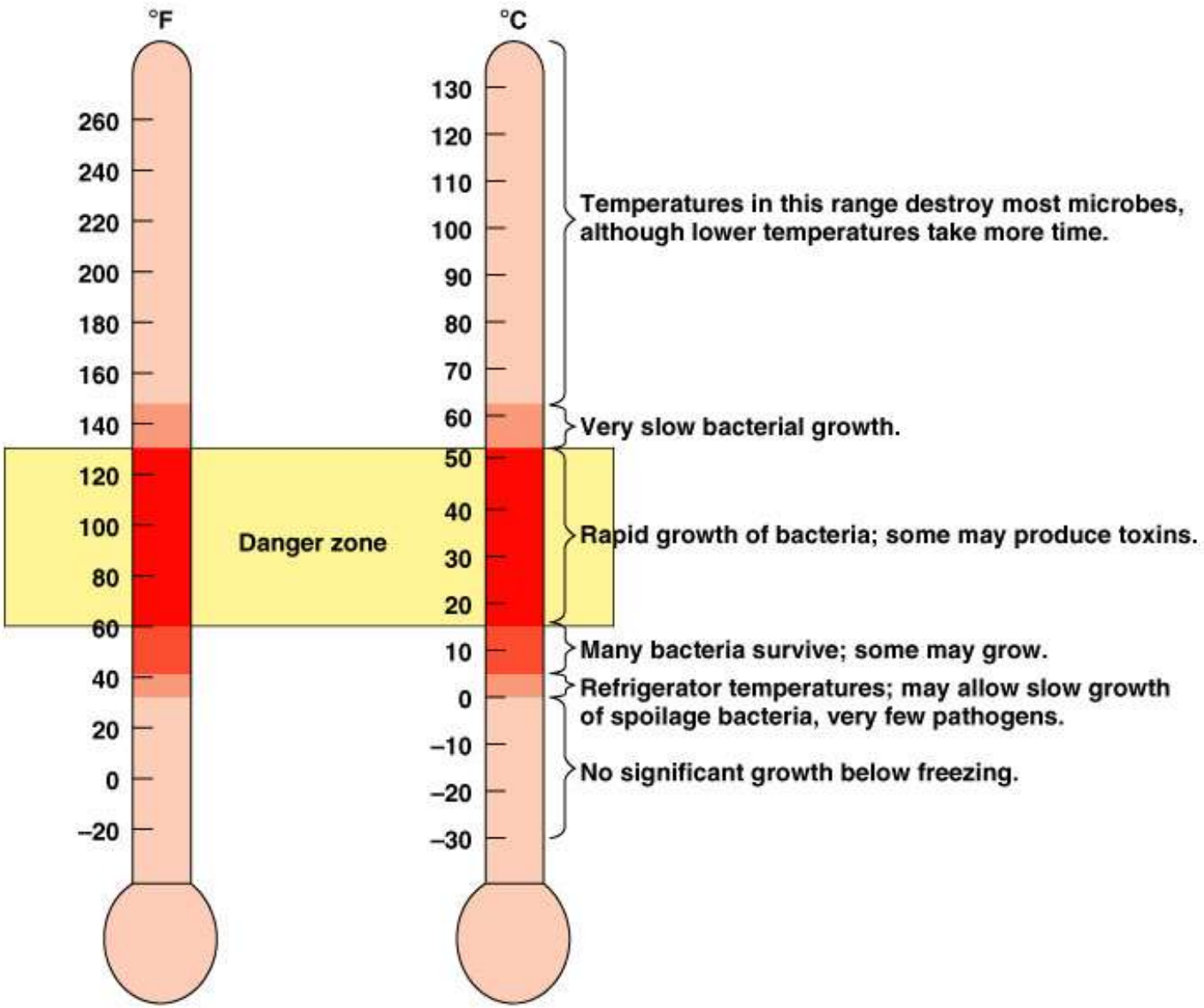


Figure 6.2

The Requirements for Growth: Physical Requirements

- pH
  - Most bacteria grow between pH 6.5 and 7.5
  - Molds and yeasts grow between pH 5 and 6
  - Acidophiles grow in acidic environments

The Requirements for Growth: Physical Requirements

- Osmotic Pressure
  - Hypertonic environments, increase salt or sugar, cause plasmolysis
  - Extreme or obligate halophiles require high osmotic pressure
  - Facultative halophiles tolerate high osmotic pressure

The Requirements for Growth: Physical Requirements

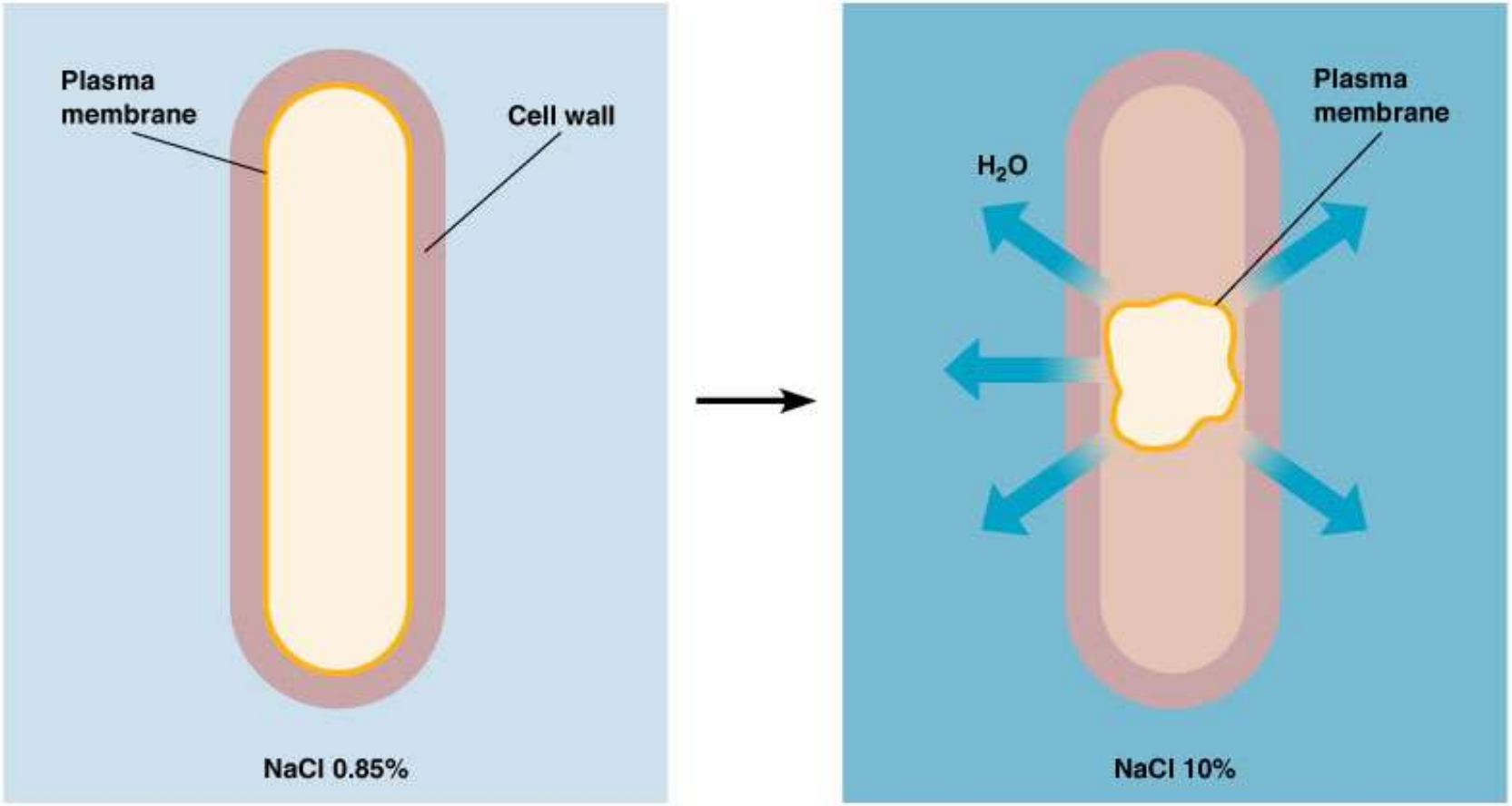


Figure 6.4

The Requirements for Growth: Chemical Requirements

- Carbon
  - Structural organic molecules, energy source
  - Chemoheterotrophs use organic carbon sources
  - Autotrophs use CO_2

The Requirements for Growth: Chemical Requirements

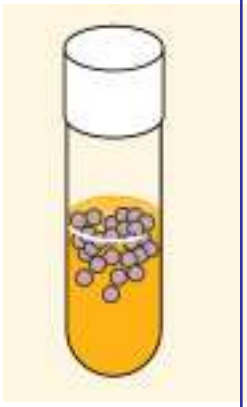
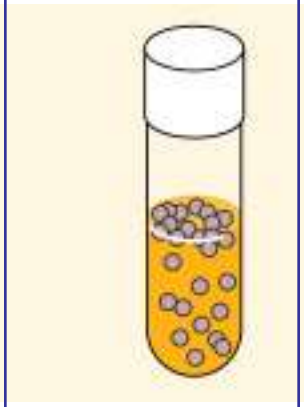
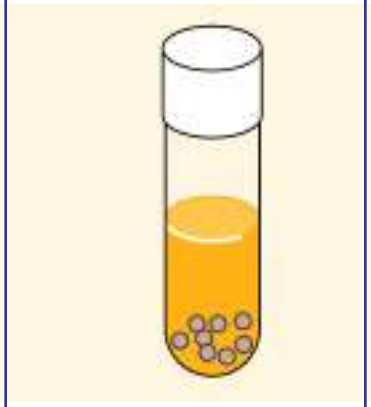
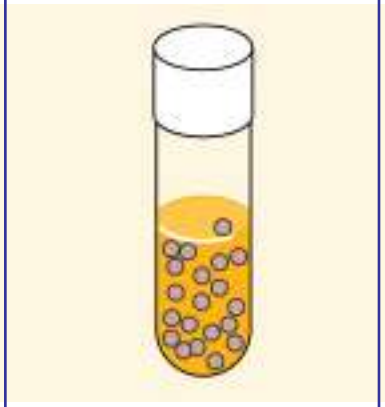
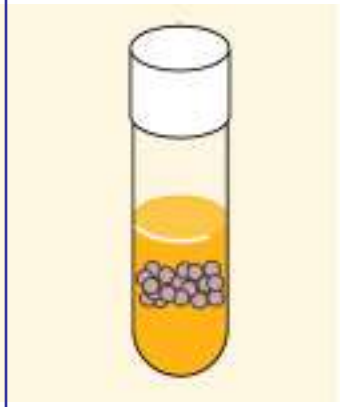
- Nitrogen
  - In amino acids, proteins
  - Most bacteria decompose proteins
  - Some bacteria use NH_4^+ or NO_3^-
  - A few bacteria use N_2 in nitrogen fixation
- Sulfur
  - In amino acids, thiamine, biotin
  - Most bacteria decompose proteins
  - Some bacteria use SO_4^{2-} or H_2S
- Phosphorus
  - In DNA, RNA, ATP, and membranes
  - PO_4^{3-} is a source of phosphorus

The Requirements for Growth: Chemical Requirements

- Trace Elements
  - Inorganic elements required in small amounts
  - Usually as enzyme cofactors

The Requirements for Growth: Chemical Requirements

- Oxygen (O_2)

obligate aerobes	Faultative anaerobes	Obligate anaerobes	Aerotolerant anaerobes	Microaerophiles
				

Toxic Forms of Oxygen

- Singlet oxygen: O_2 boosted to a higher-energy state
- Superoxide free radicals: O_2^-

$$O_2^- + 2\,H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

- Peroxide anion: O_2^{2-}

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

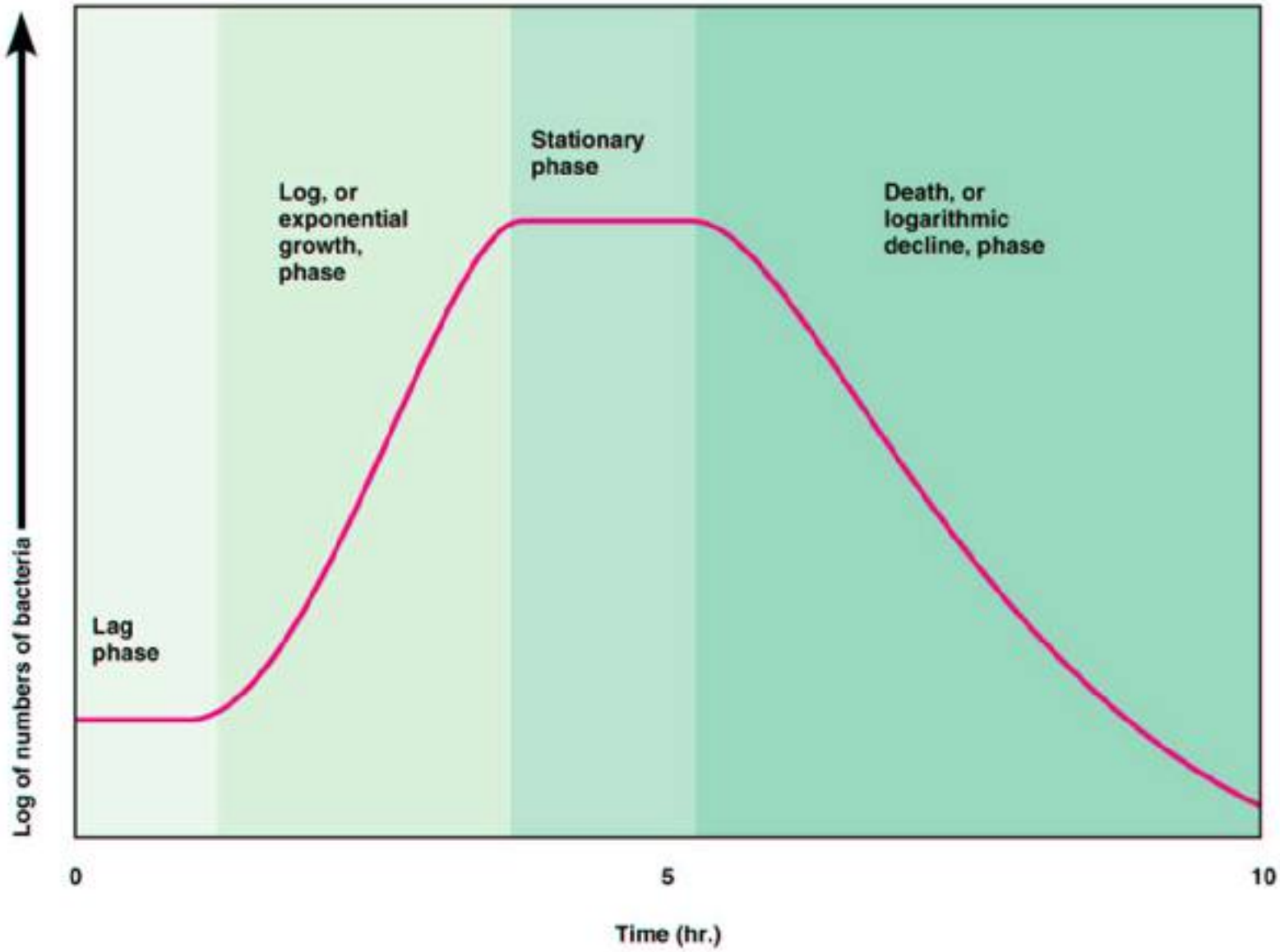
$$H_2O_2 + 2\,O^+ \xrightarrow{\text{peroxidase}} 2\,H_2O$$

- Hydroxyl radical (OH•)

The Requirements for Growth: Chemical Requirements

- Organic Growth Factors
  - Organic compounds obtained from the environment
  - Vitamins, amino acids, purines, pyrimidines

Growth cycle



Generation time

- time for bacterial mass to double

- Example

  100 bacteria present at time 0

  If generation time is 2 hr

  After 8 hr mass = 100×2^4

Fermentation of Sugars

- Breakdown of sugars to pyruvic acid and then to lactic acid (glycolytic cycle)
- *N. meningitidis* vs. *N. gonorrhoeae* (glucose & maltose)
- L.F. as *E. coli* has beta-galctosidase
- If oxygen is present, pyruvate enters Krebs cycle and is metabolized to CO2 & H2O.
- Aerobes as *P. aeruginosa* produce metabolites that enter Krebs cycle by deamination of a.a.
- In clinical lab., pH indicators as phenol red are used.

Aerobic & Anaerobic Growth

- Obligate aerobes
- Facultative anaerobes
- Obligate anaerobes

Iron Metabolism

- Iron in form of ferric ion is required for growth
- In body, iron mostly sequestered (transferrin)
- Siderophores can capture iron (chelating agent) (e.g. enterobactin).

Culture Media

- Culture Medium: Nutrients prepared for microbial growth
- Sterile: No living microbes
- Inoculum: Introduction of microbes into medium
- Culture: Microbes growing in/on culture medium

Agar

- Complex polysaccharide
- Used as solidifying agent for culture media in Petri plates, slants, and deeps
- Generally not metabolized by microbes
- Liquefies at 100°C
- Solidifies ~40°C

Culture Media

- Chemically Defined Media: Exact chemical composition is known
- Complex Media: Extracts and digests of yeasts, meat, or plants
  - Nutrient broth
  - Nutrient agar

Culture Media

TABLE 6.2 **A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *E. coli***

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

TABLE 6.4 **Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria**

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

Table 6.2 & 6.4

Anaerobic Culture Methods

- Reducing media
- **Reducing media are used for growing anaerobic bacteria in the laboratory. Since obligate anaerobes do not grow in the presence of oxygen, this type of media uses a chemical substance, such as thioglycolate, to remove molecular oxygen that is dissolved in the media**
  - Contain chemicals (thioglycollate or oxyrase) that combine O_2
  - Heated to drive off O_2

Anaerobic Culture Methods

- Anaerobic jar

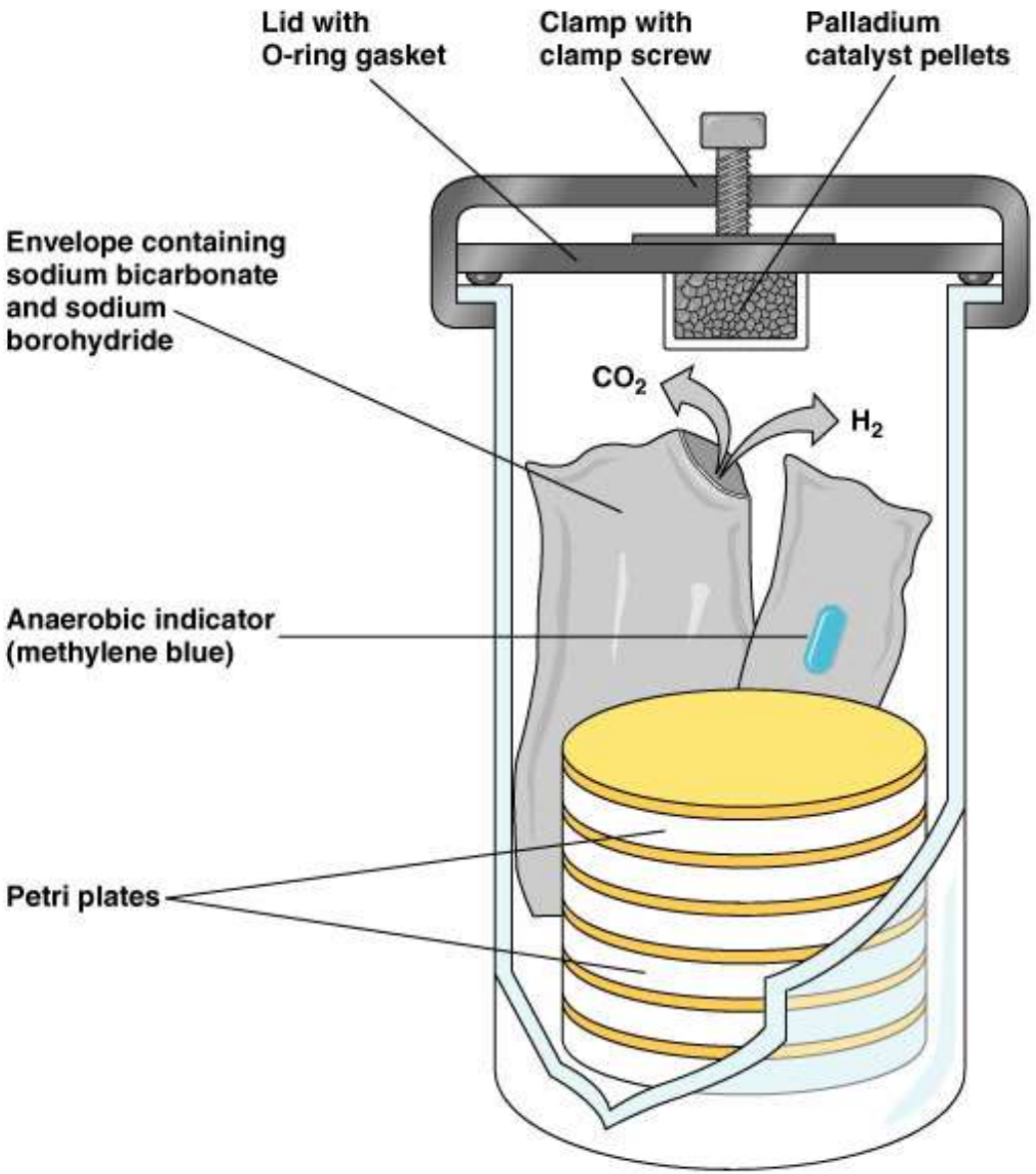


Figure 6.5

Anaerobic Culture Methods

- Anaerobic chamber

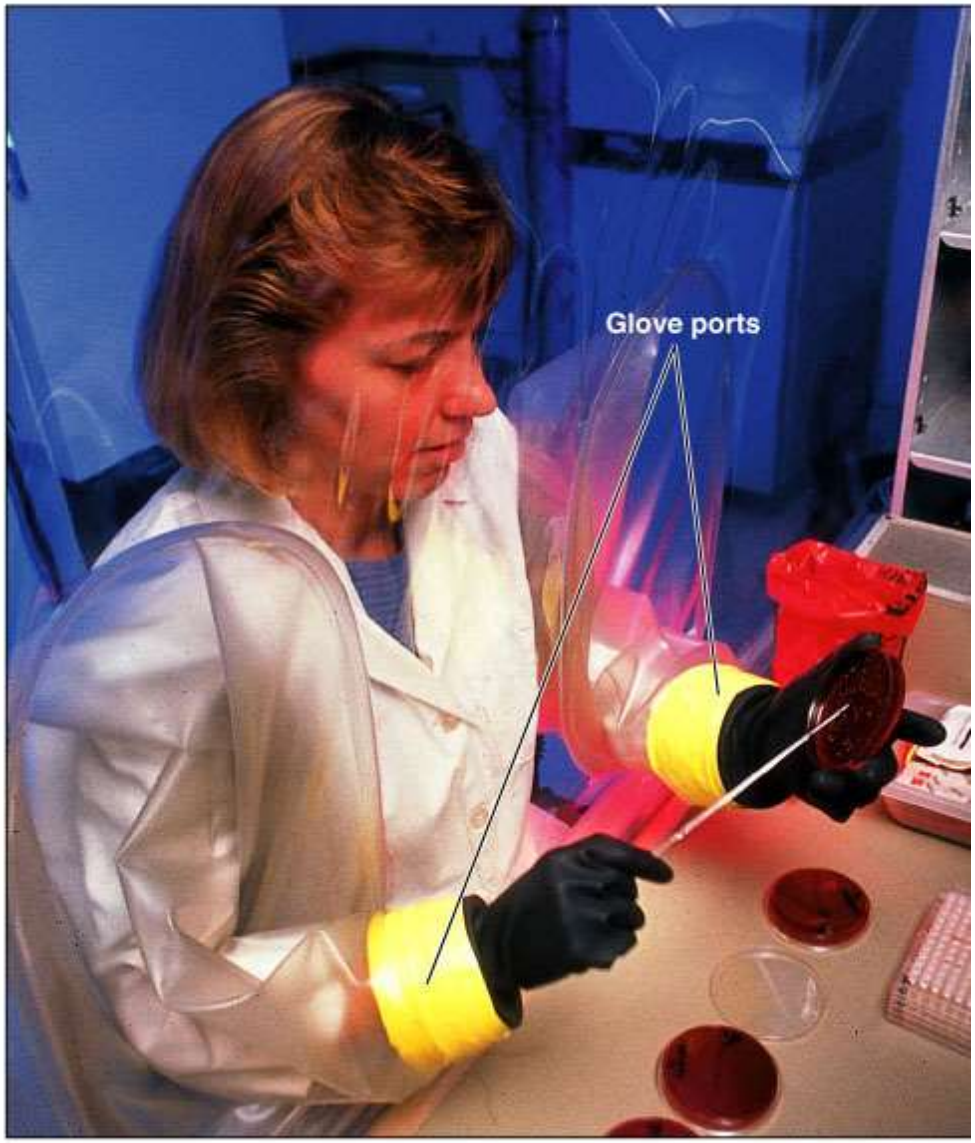
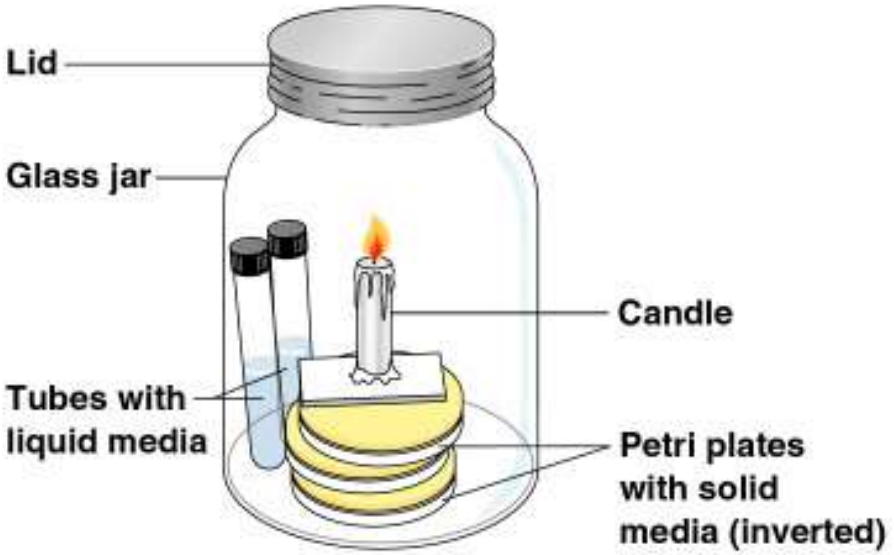


Figure 6.6

Capnophiles require high CO_2

- Candle jar



- CO_2-packet

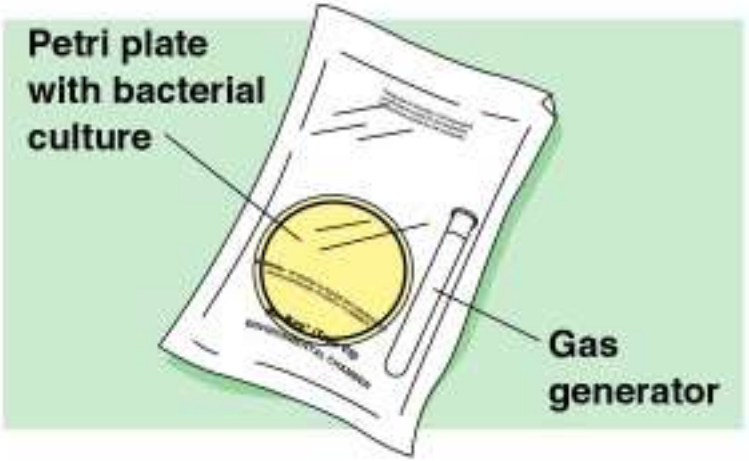


Figure 6.7

Selective Media

- Suppress unwanted microbes and encourage desired microbes.

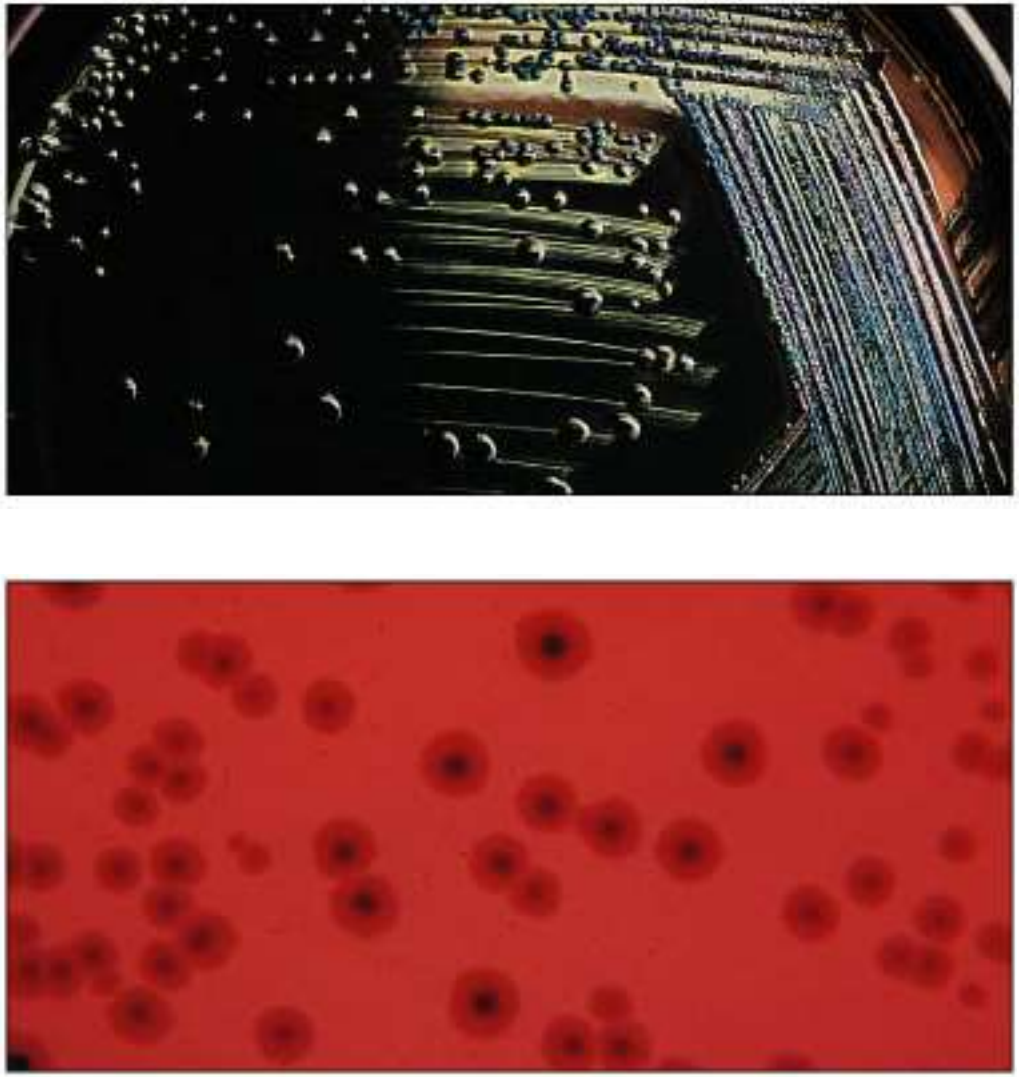


Figure 6.9b, c

Differential Media

- Make it easy to distinguish colonies of different microbes.

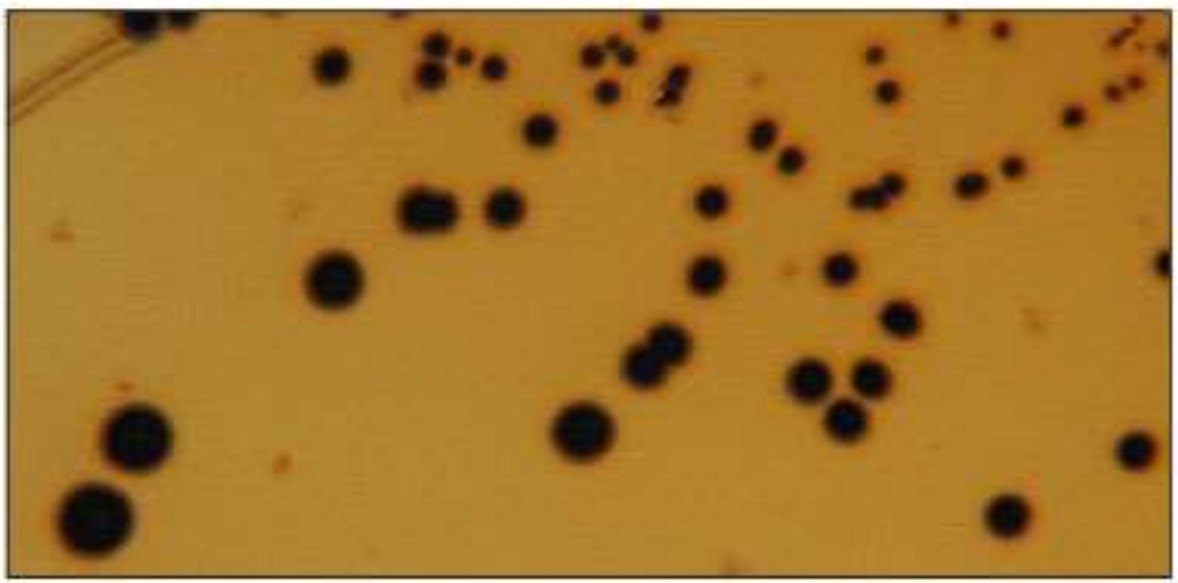


Figure 6.9a

Enrichment Media

- Encourages growth of desired microbe
- Assume a soil sample contains a few phenol-degrading bacteria and thousands of other bacteria
  - Inoculate phenol-containing culture medium with the soil and incubate
  - Transfer 1 ml to another flask of the phenol medium and incubate
  - Transfer 1 ml to another flask of the phenol medium and incubate
  - Only phenol-metabolizing bacteria will be growing

- A pure culture contains only one species or strain
- A colony is a population of cells arising from a single cell or spore or from a group of attached cells
- A colony is often called a colony-forming unit (CFU)

Streak Plate

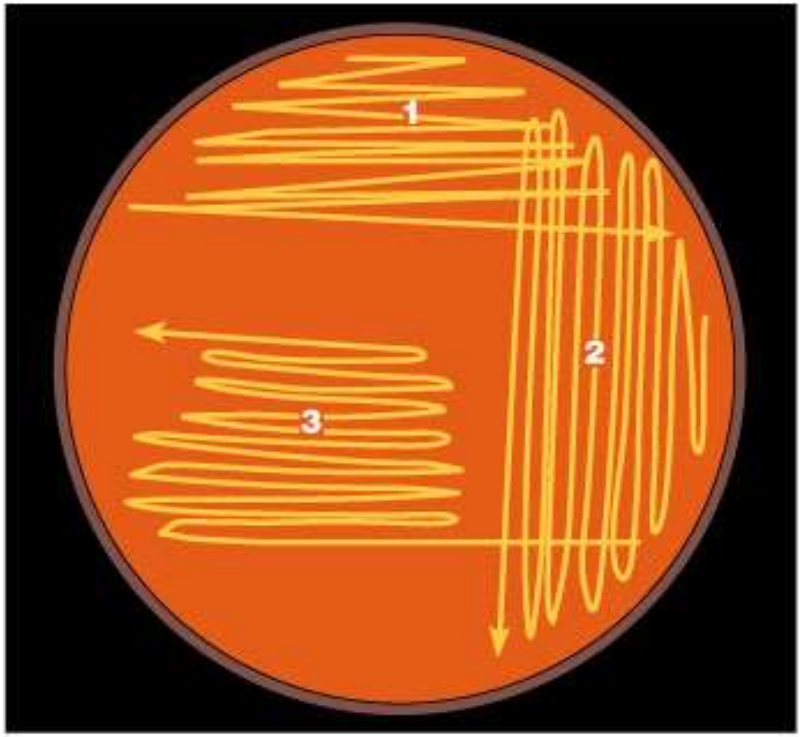


Figure 6.10a, b

TORTORA • FUNKE • CASE

Microbiology

AN INTRODUCTION

EIGHTH EDITION

Chapter 4

Microbial Genetics

PowerPoint® Lecture Slide Presentation prepared by Christine L. Case

Terminology

- Genetics — Study of what genes are, how they carry information, how information is expressed, and how genes are replicated
- Gene — Segment of DNA that encodes a functional product, usually a protein

Terminology

- Genome — All of the genetic material in a cell
- Genomics — Molecular study of genomes
- Genotype — Genes of an organism
- Phenotype — Expression of the genes

E. coli

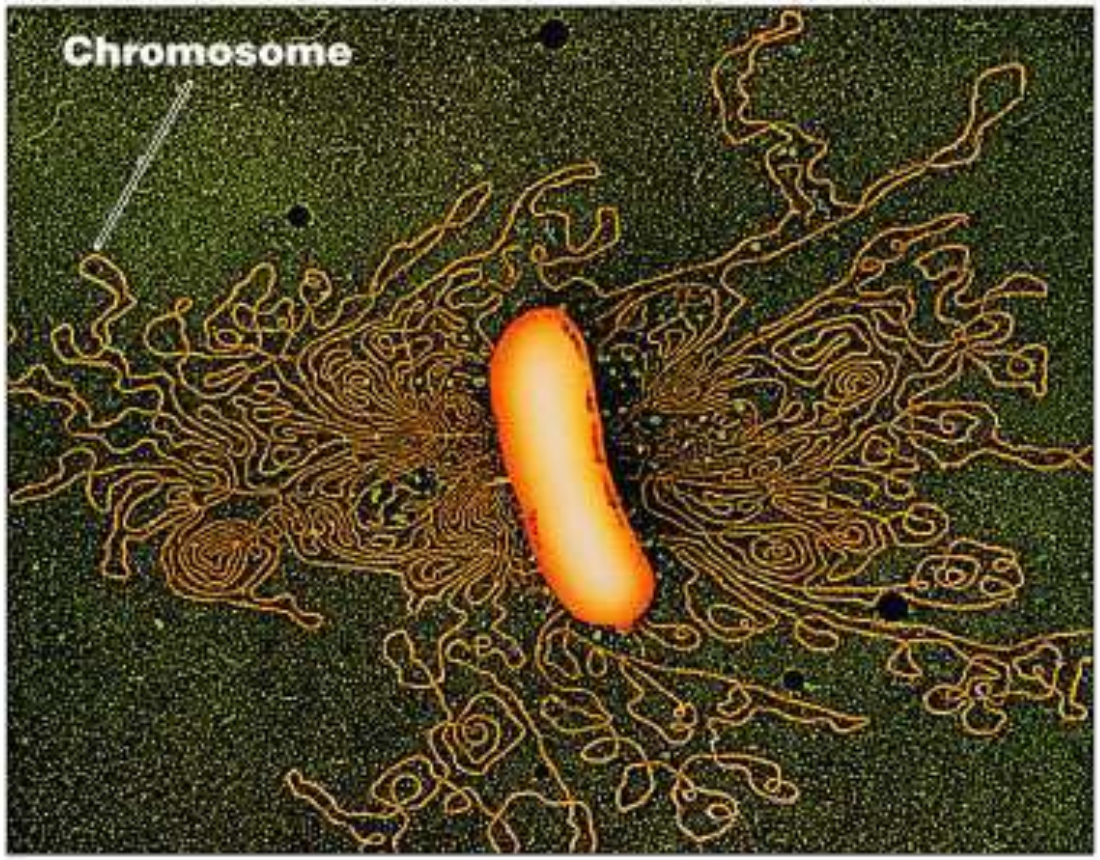


Figure 8.1a

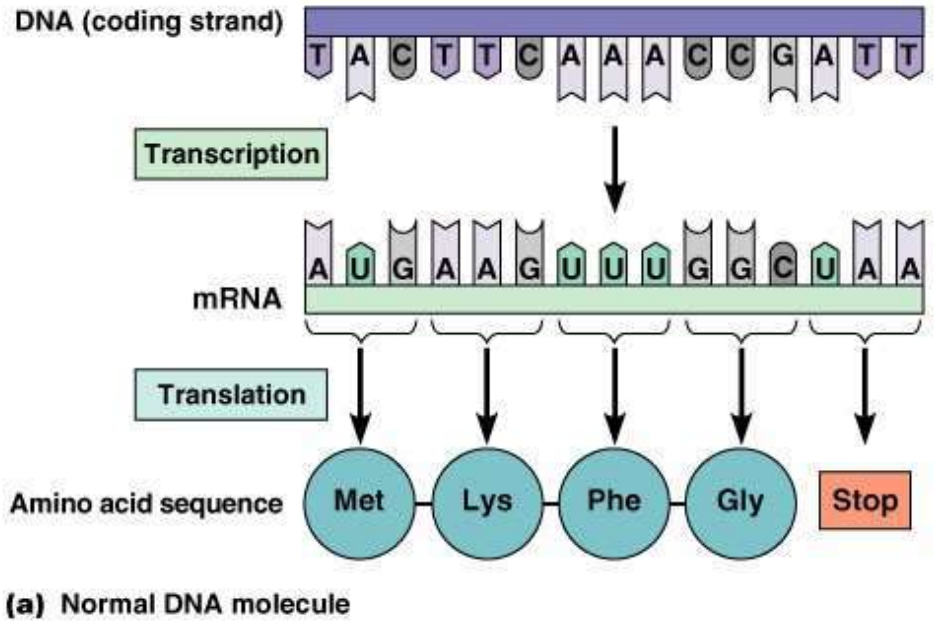
Mutation

- Change in the genetic material
- Mutations may be neutral, beneficial, or harmful
- Mutagen: Agent that causes mutations
- Spontaneous mutations: Occur in the absence of a mutagen

Mutation

- Base substitution (point mutation)
- Missense mutation

- Change in one base
- Result in change in amino acid



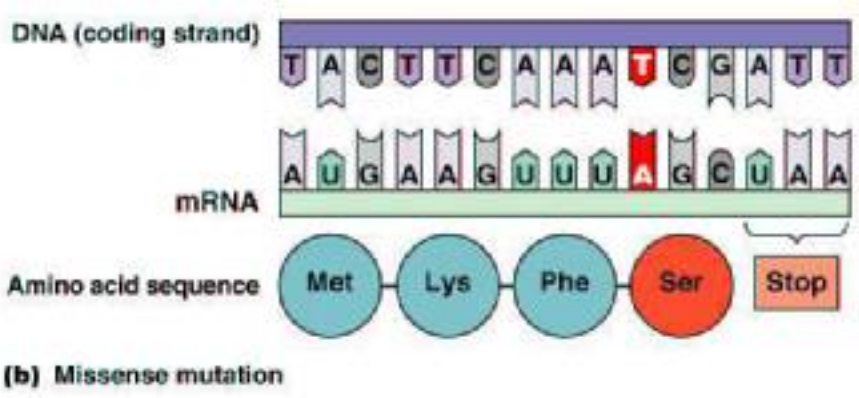
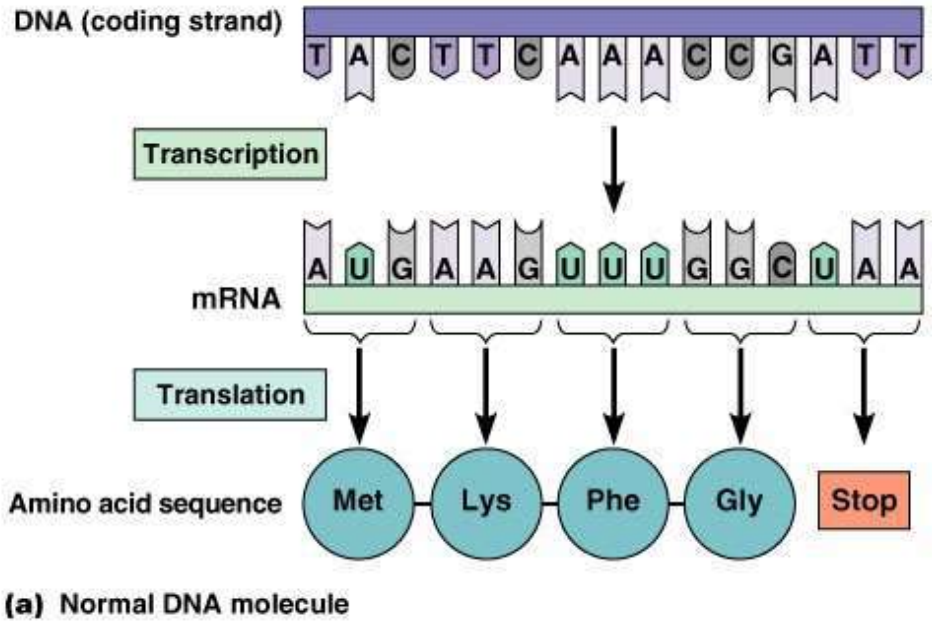


Figure 8.17a, b

Mutation

- Nonsense mutation
- Results in a nonsense codon



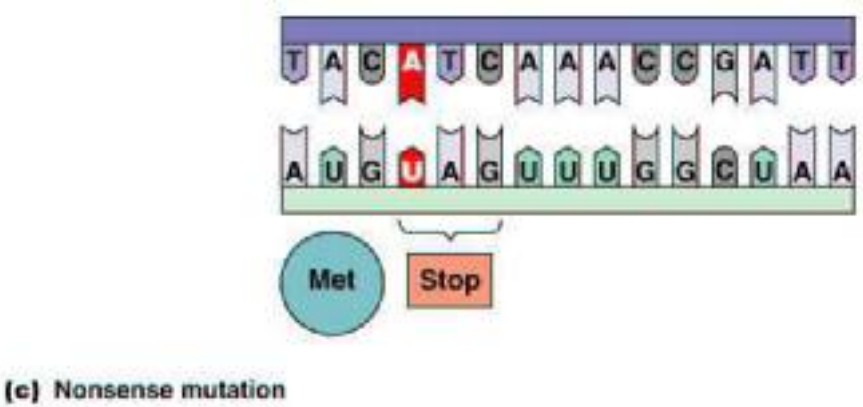
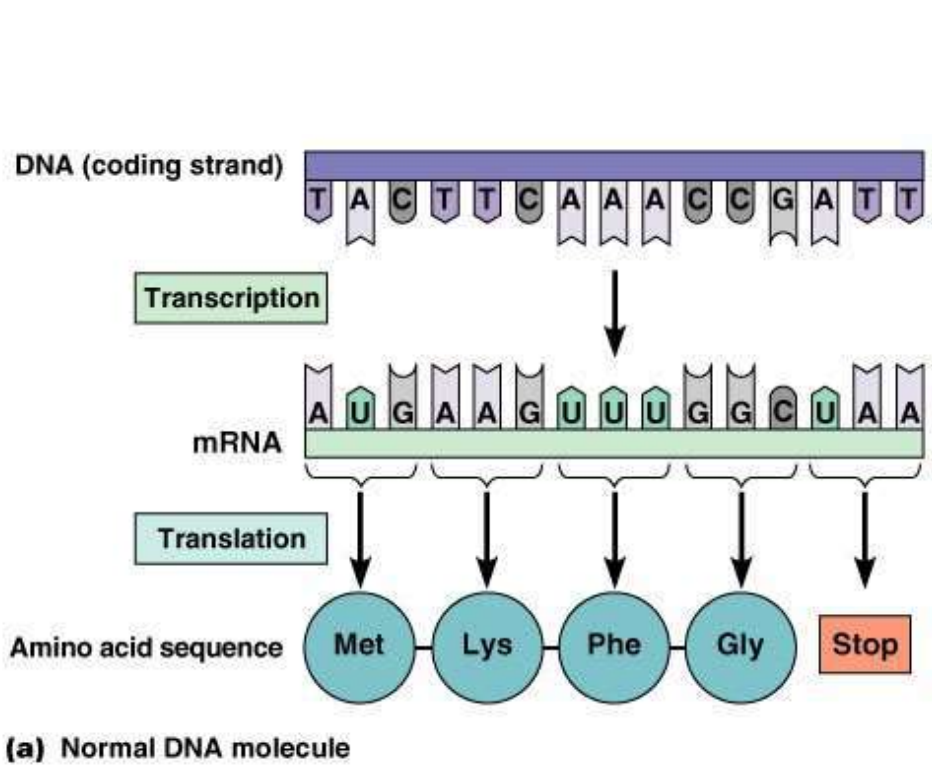


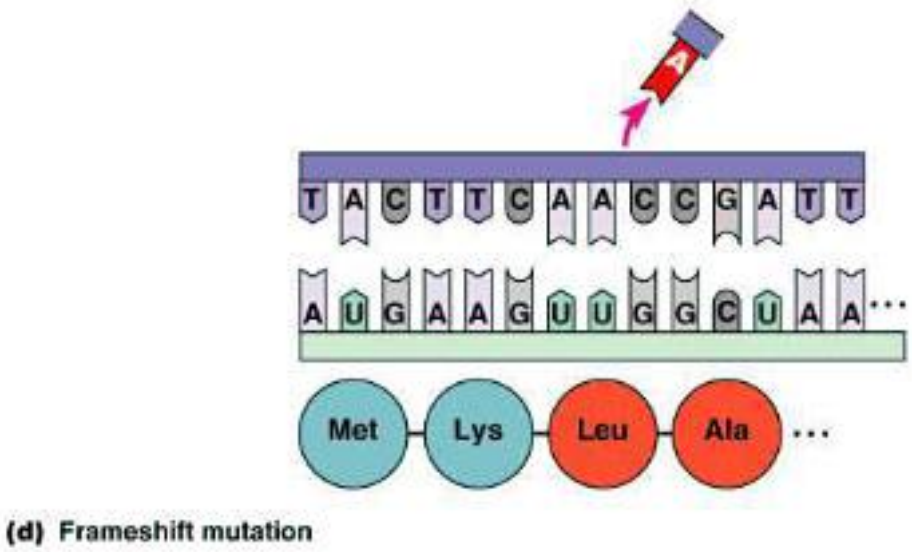
Figure 8.17a, c

Mutation

- Frameshift mutation
- Insertion or deletion of one or more nucleotide pairs



(a) Normal DNA molecule



(d) Frameshift mutation

Figure 8.17a, d

Mutation

- Third type of mutation is transposons or insertion sequences.
- Mutations can be caused by chemical, radiation or viruses.
  - - nitrous acid & alkylating agents (A-T) to (A-C)
  - - 5-bromouracil (A-T) to (G-C)
  - - benzpyrene in tobacco smoke (frameshift mutation)

Mutation

- Ionizing radiation (X rays and gamma rays) causes the formation of ions that can react with nucleotides and the deoxyribose-phosphate backbone.
- Nucleotide excision repairs mutations
- Mu (mutator bacteriophage)
- Conditional lethal mutations: (are of medical interest)

Mutation

- UV radiation causes thymine dimers
- Light-repair separates thymine dimers

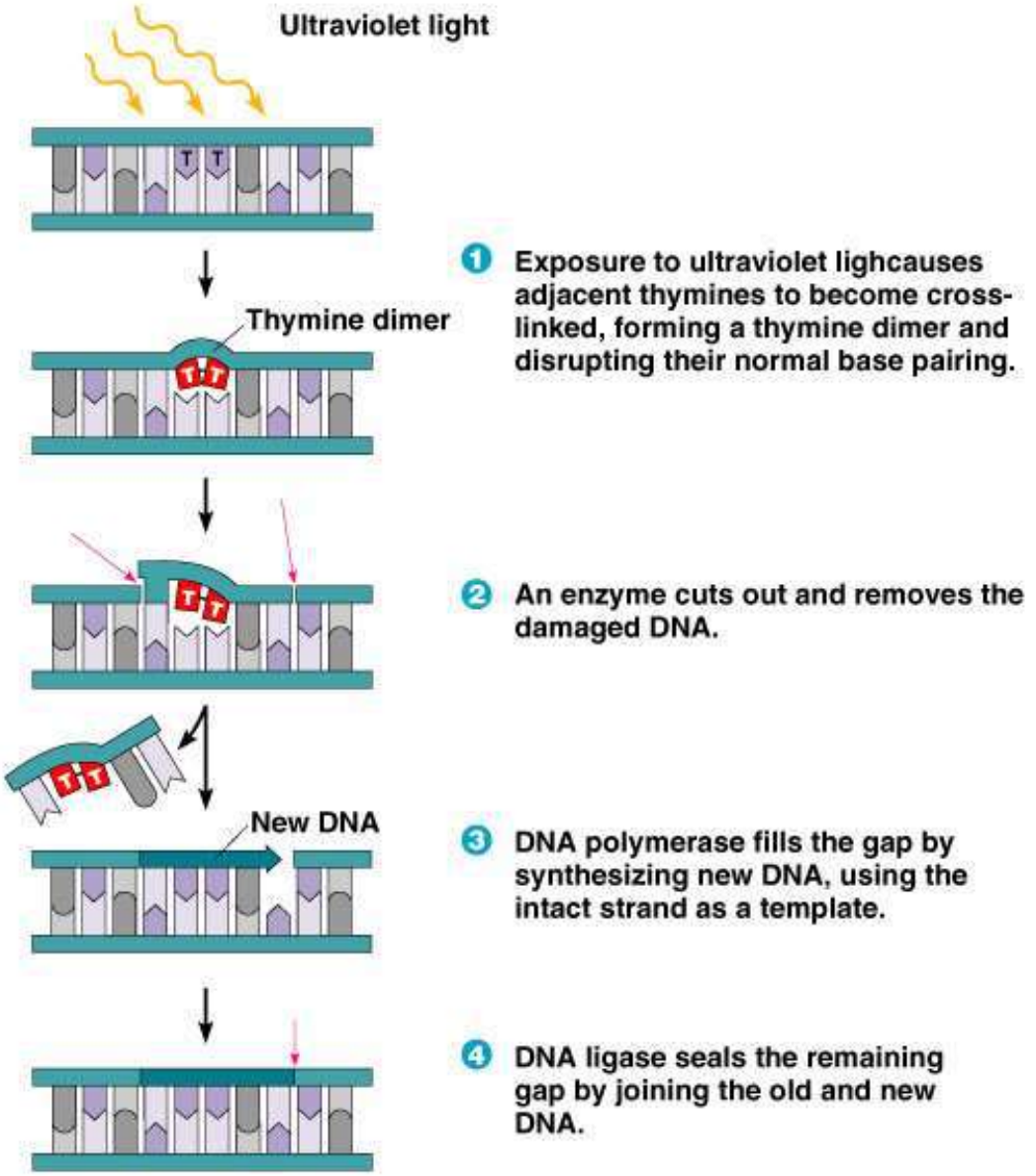


Figure 8.20

The Frequency of Mutation

- Spontaneous mutation rate = 1 in 10^9 replicated base pairs or 1 in 10^6 replicated genes
- Mutagens increase to 10^{-5} or 10^{-3} per replicated gene

Selection

- Positive (direct) selection detects mutant cells because they grow or appear different.
- Negative (indirect) selection detects mutant cells because they do not grow.

Replica Plating

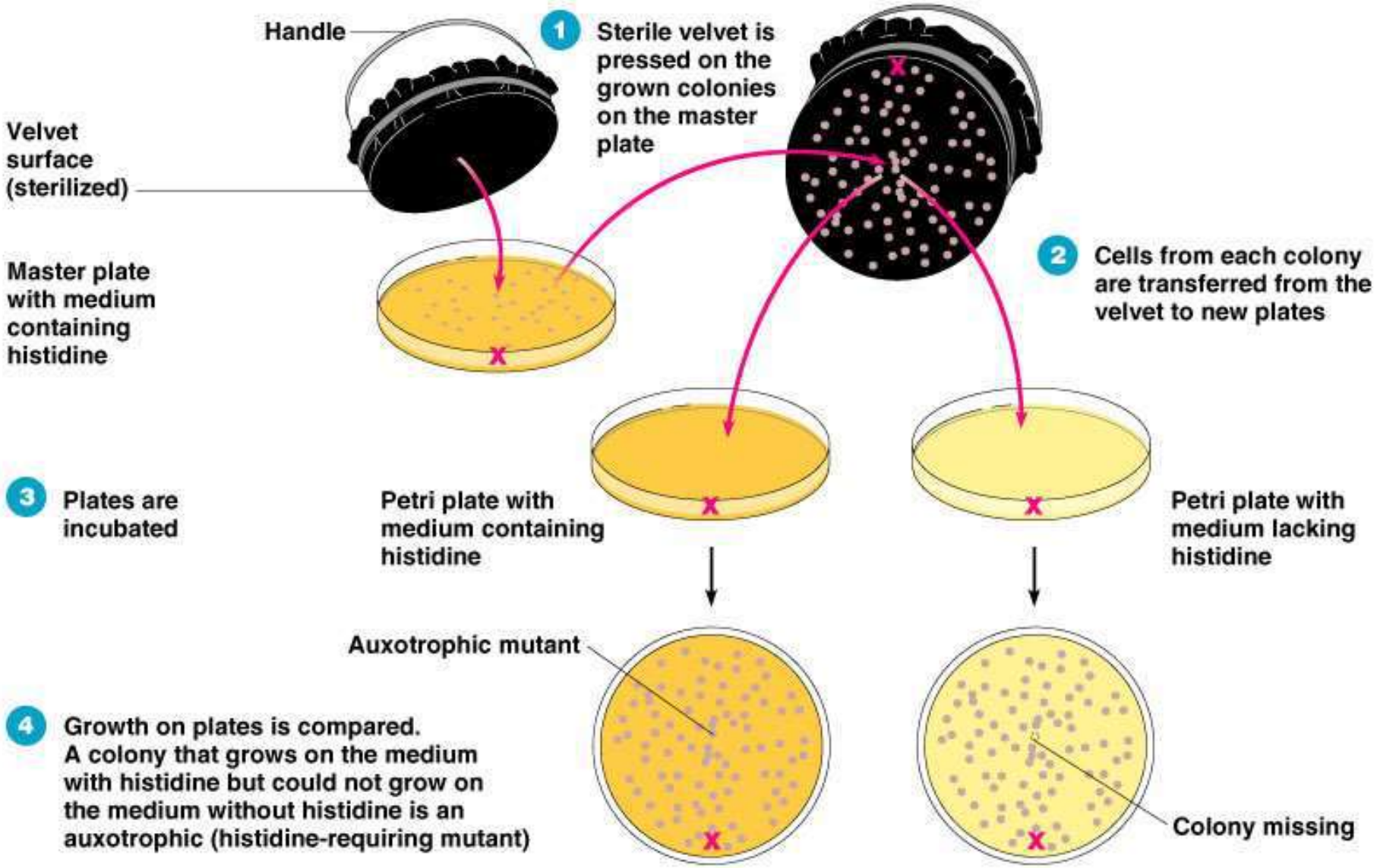


Figure 8.21

Genetic Transfer and Recombination

- Vertical gene transfer
  - Occurs during reproduction, between generations of cells
- Horizontal gene transfer
  - Transfer of genes between cells of the same generation

Transformation

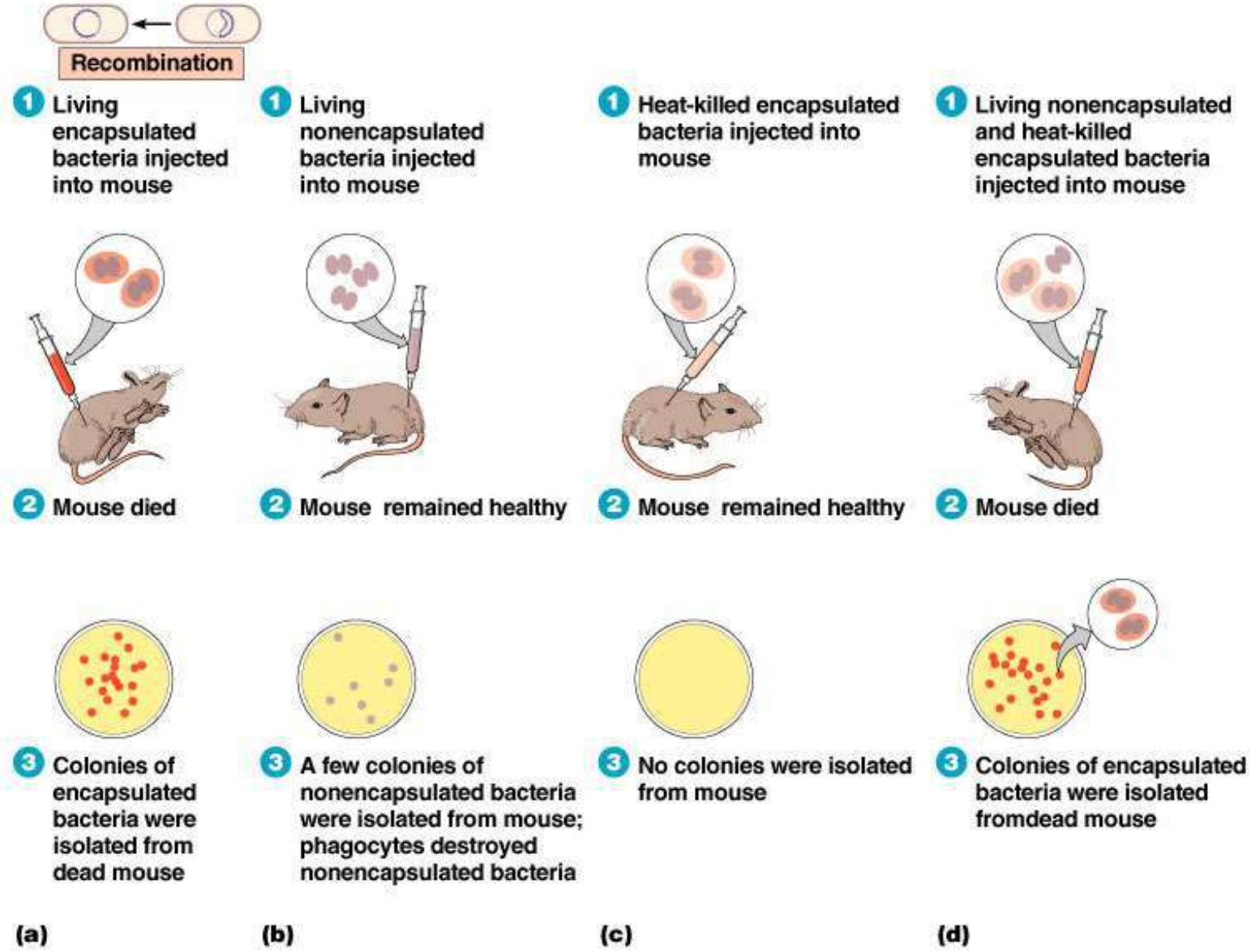


Figure 8.24

Recombination

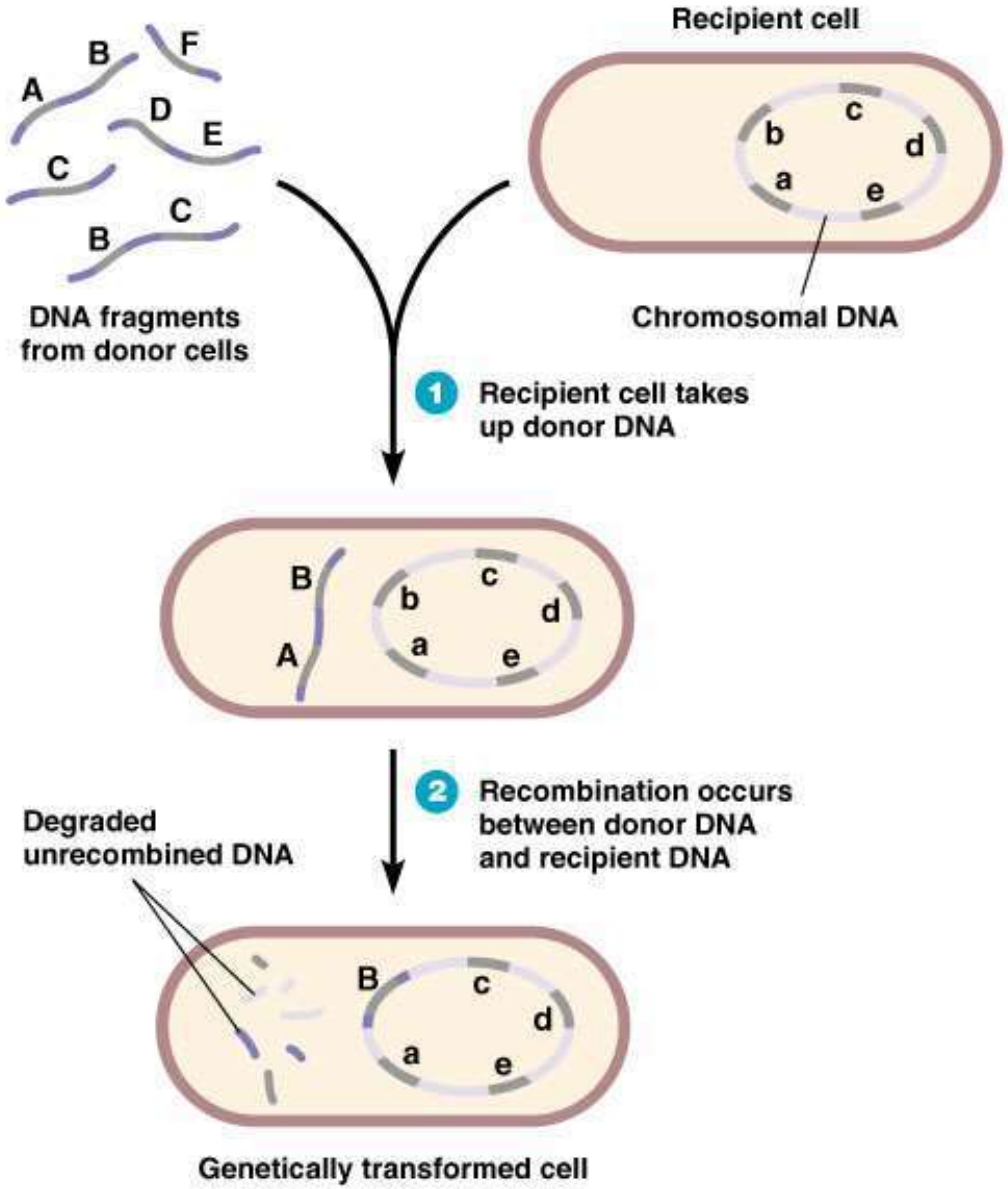
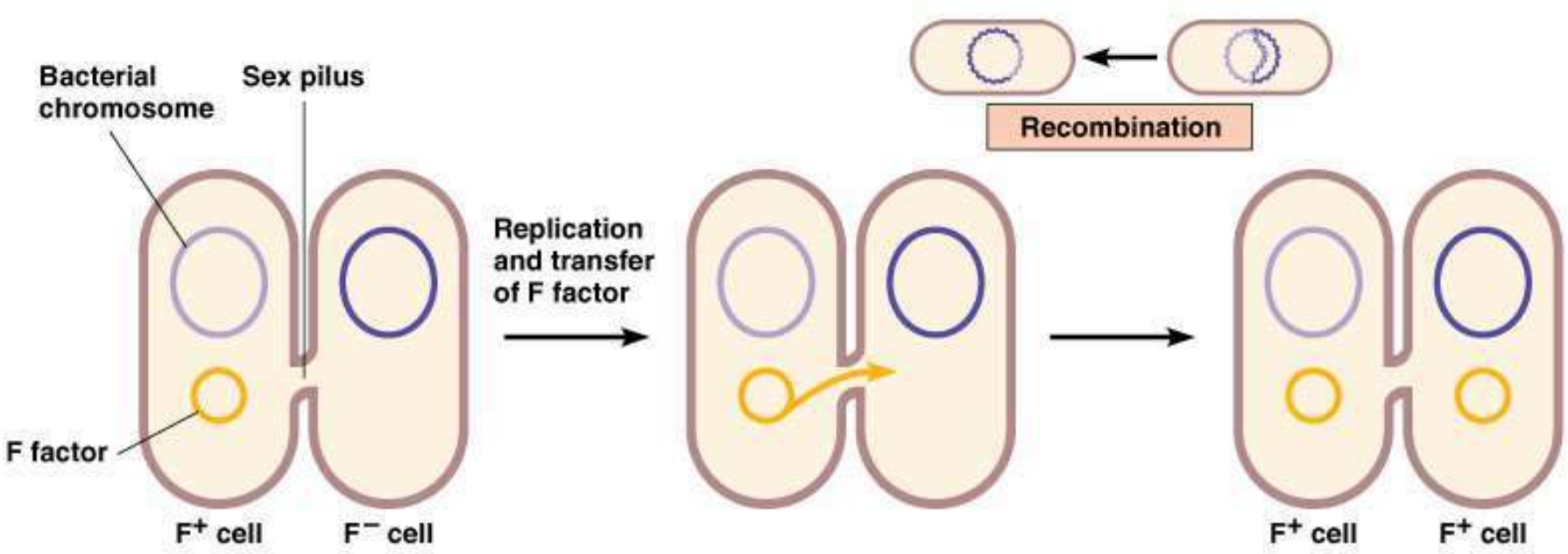


Figure 8.25

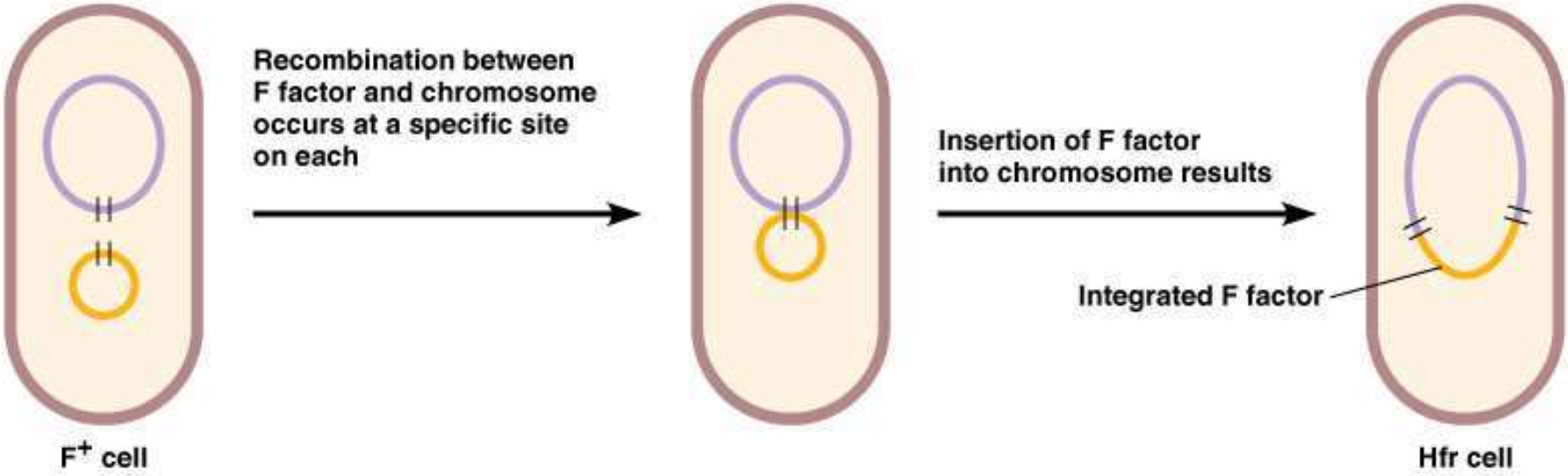
Conjugation



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted into an F^+ cell.

Figure 8.27a

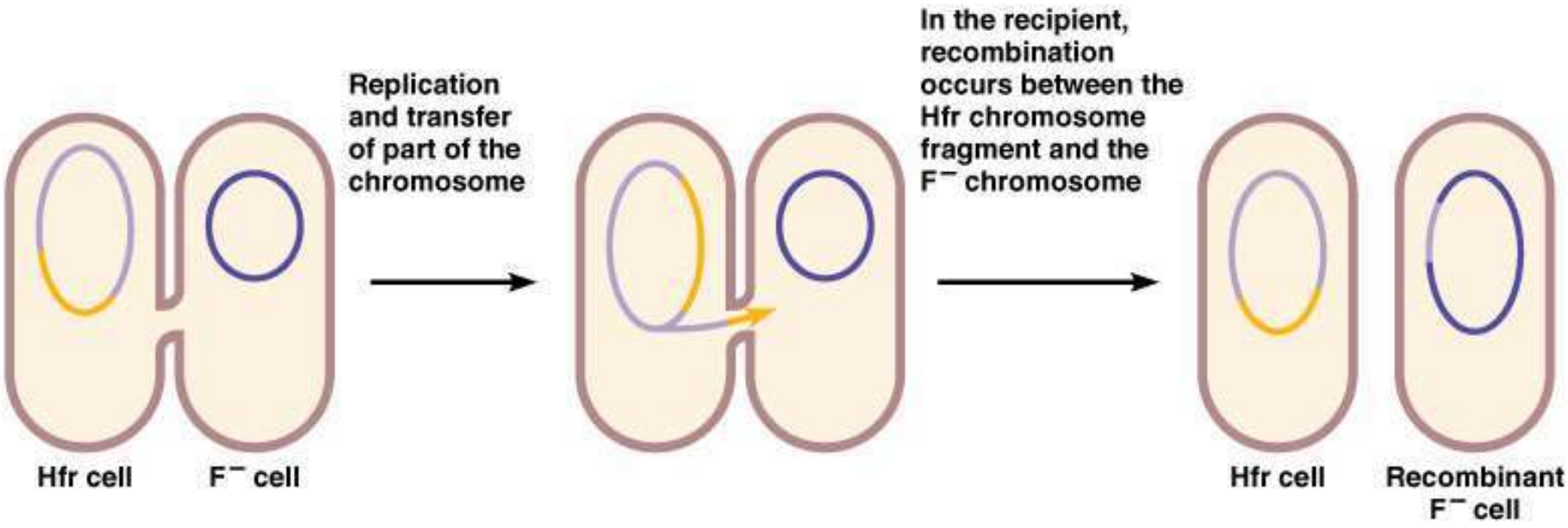
Conjugation



(b) When an F factor becomes integrated into the chromosome of an F^+ cell, it makes the cell a high frequency of recombination (Hfr) cell.

Figure 8.27b

Conjugation



(c) When an Hfr donor passes a portion of its chromosome into an F^- recipient, a recombinant F^- cell results.

Figure 8.27c

Genetic Recombination

- Exchange of genes between two DNA molecules
  - Crossing over occurs when two chromosomes break and rejoin

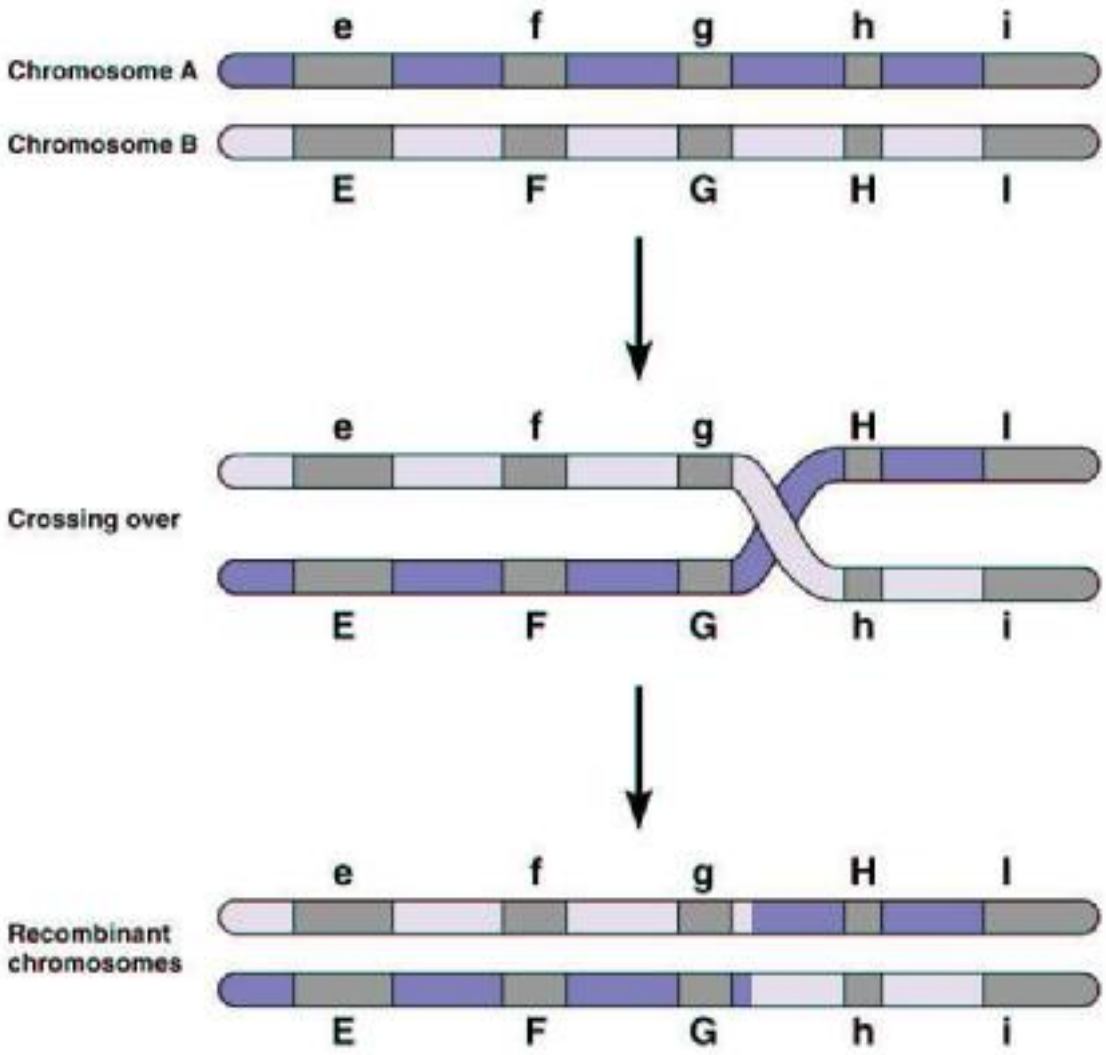


Figure 8.23

Transduction

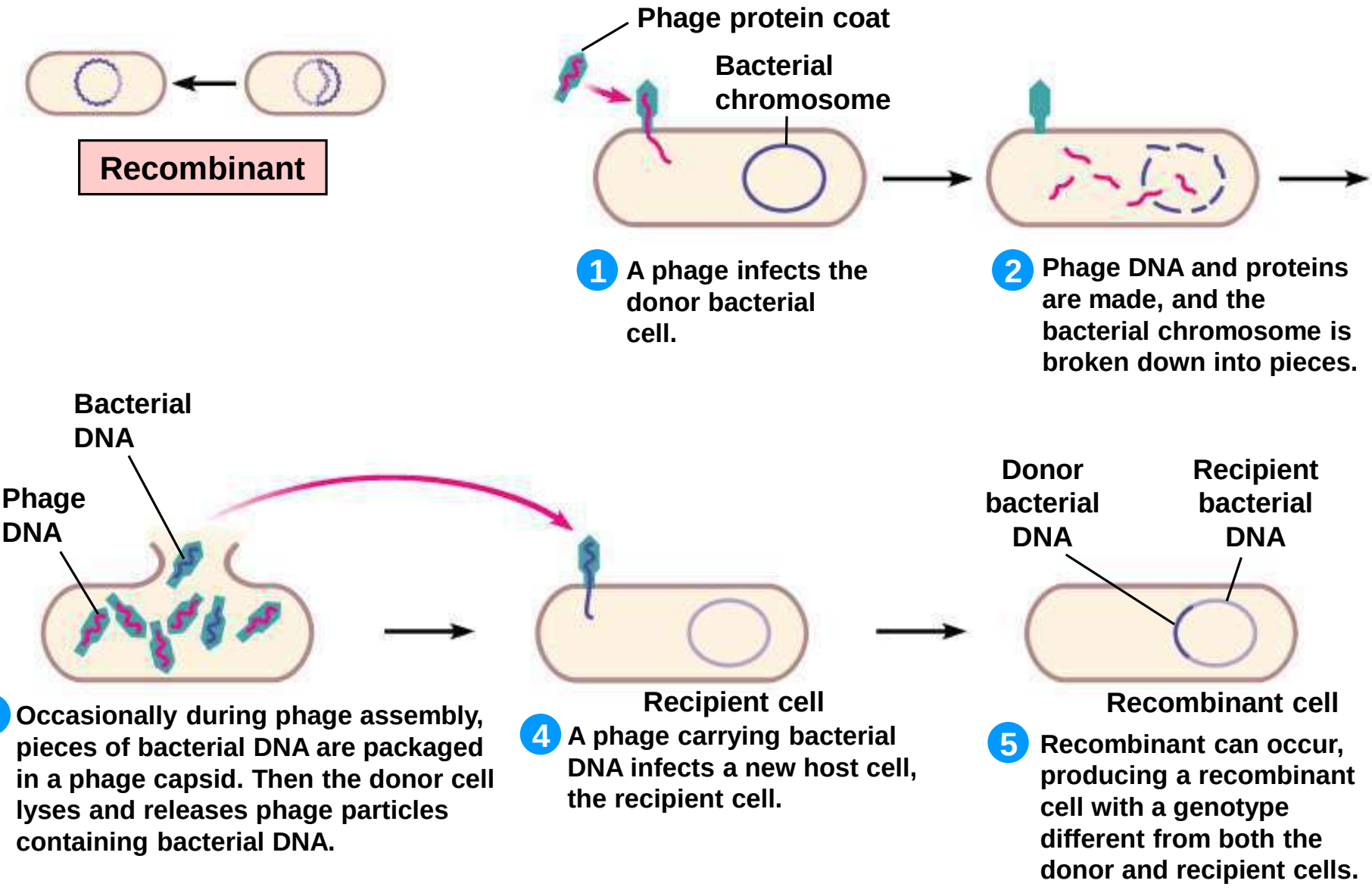
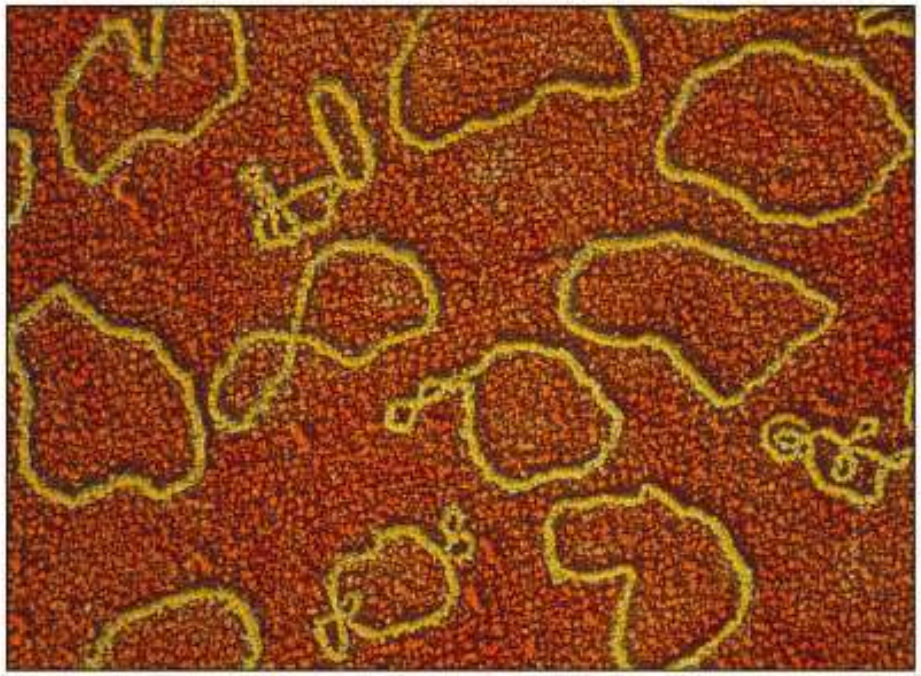


Figure 8.28

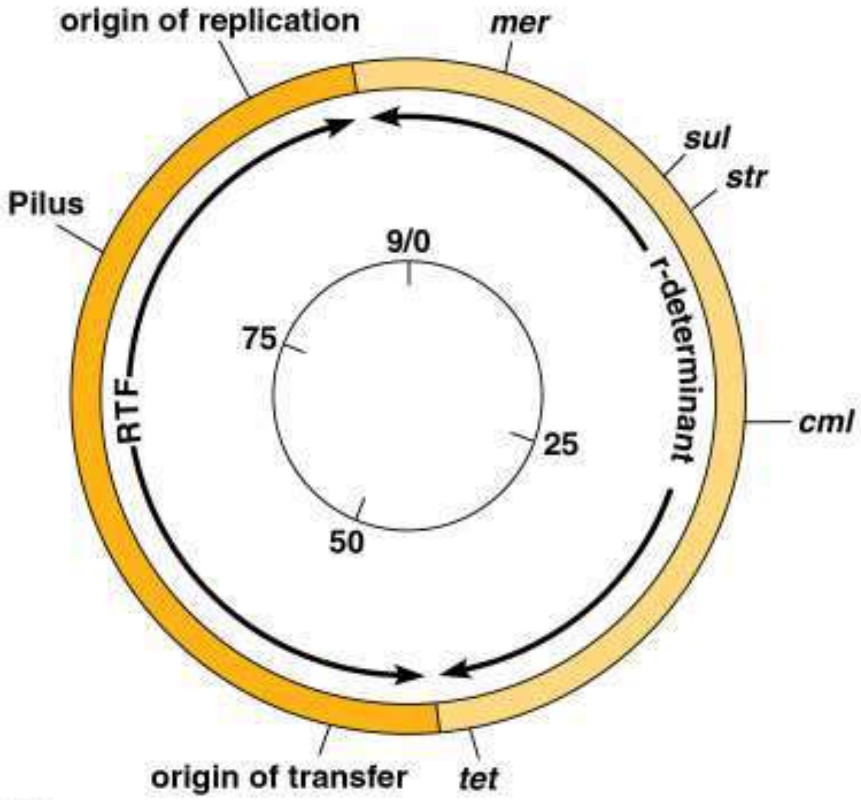
Plasmids

• Conjugative plasmid	Carries genes for sex pili and transfer of the plasmid
• Dissimilation plasmids	Encode enzymes for catabolism of unusual compounds
• R factors	Encode antibiotic resistance

Plasmids



(a)



(b)

Figure 8.29

Transposons

- Segments of DNA that can move from one region of DNA to another
- Contain insertion sequences for cutting and resealing DNA (transposase)
- Complex transposons carry other genes

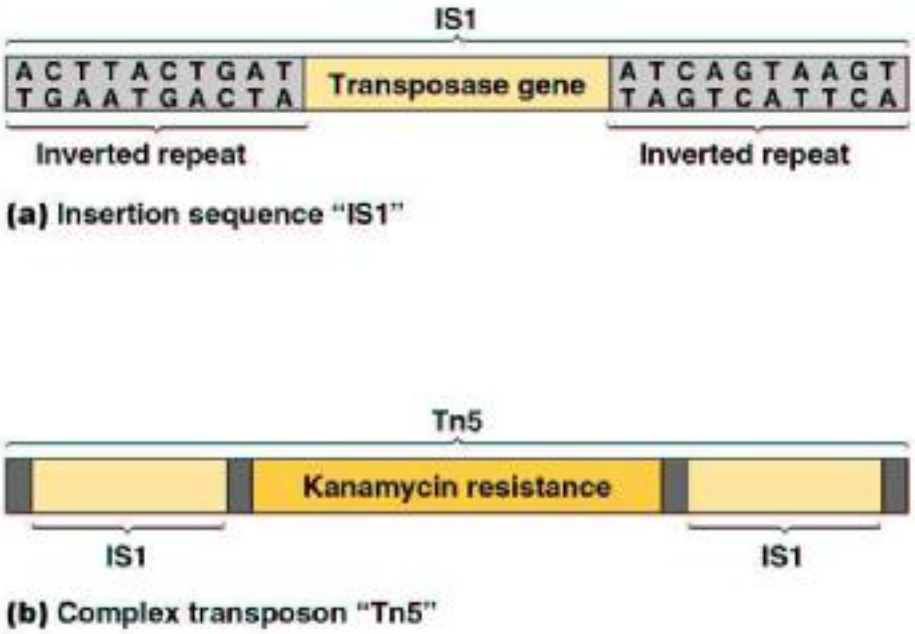


Figure 8.30a, b

Regulation of Bacterial Gene Expression

- Constitutive enzymes are expressed at a fixed rate
- Other enzymes are expressed only as needed
  - Repressible enzymes
  - Inducible enzymes

Repression

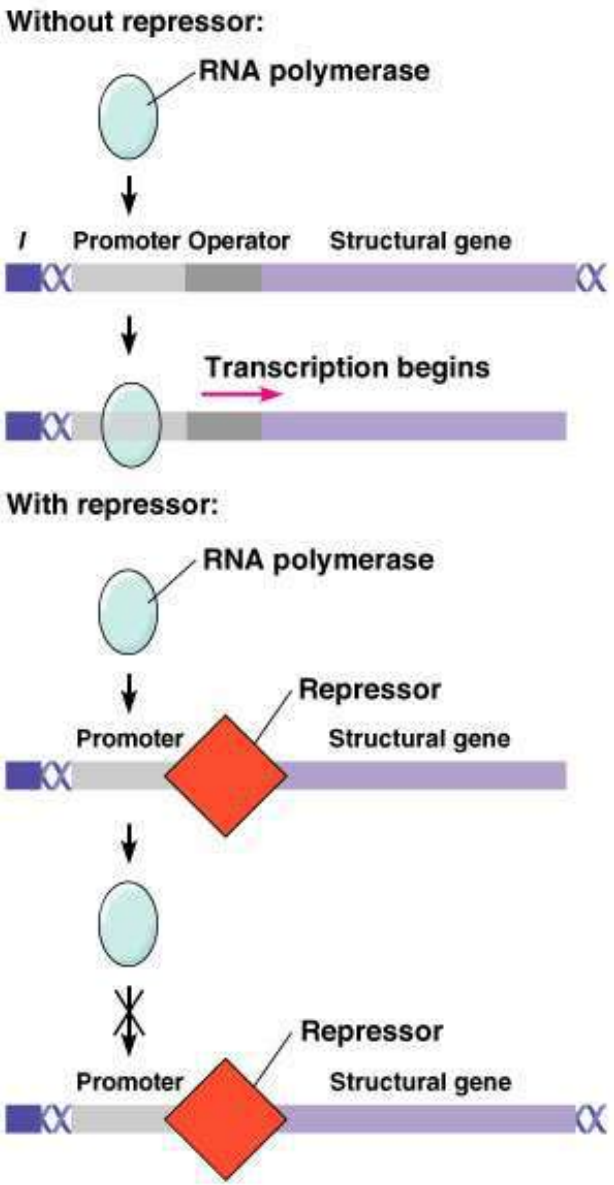
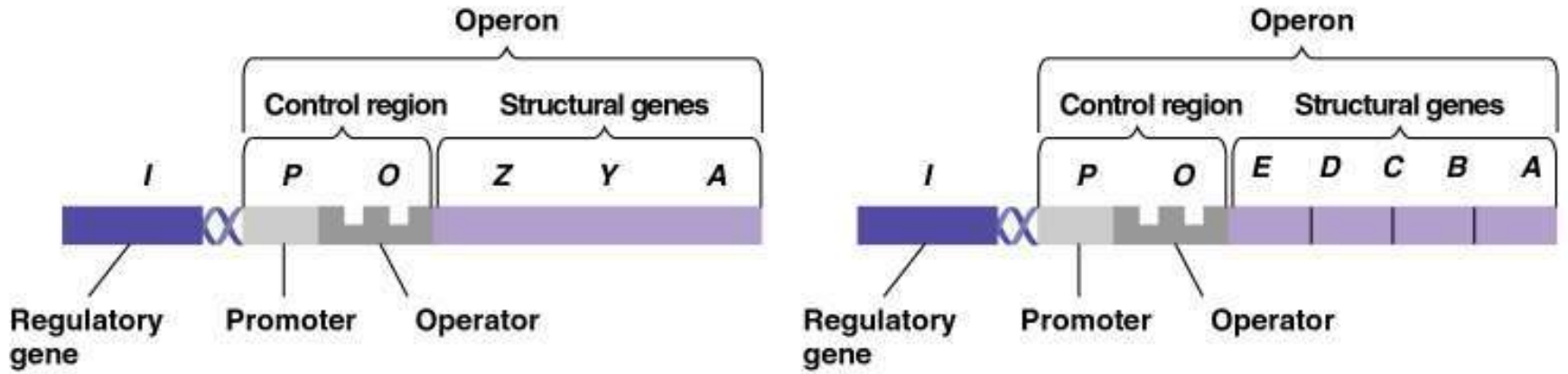


Figure 8.13

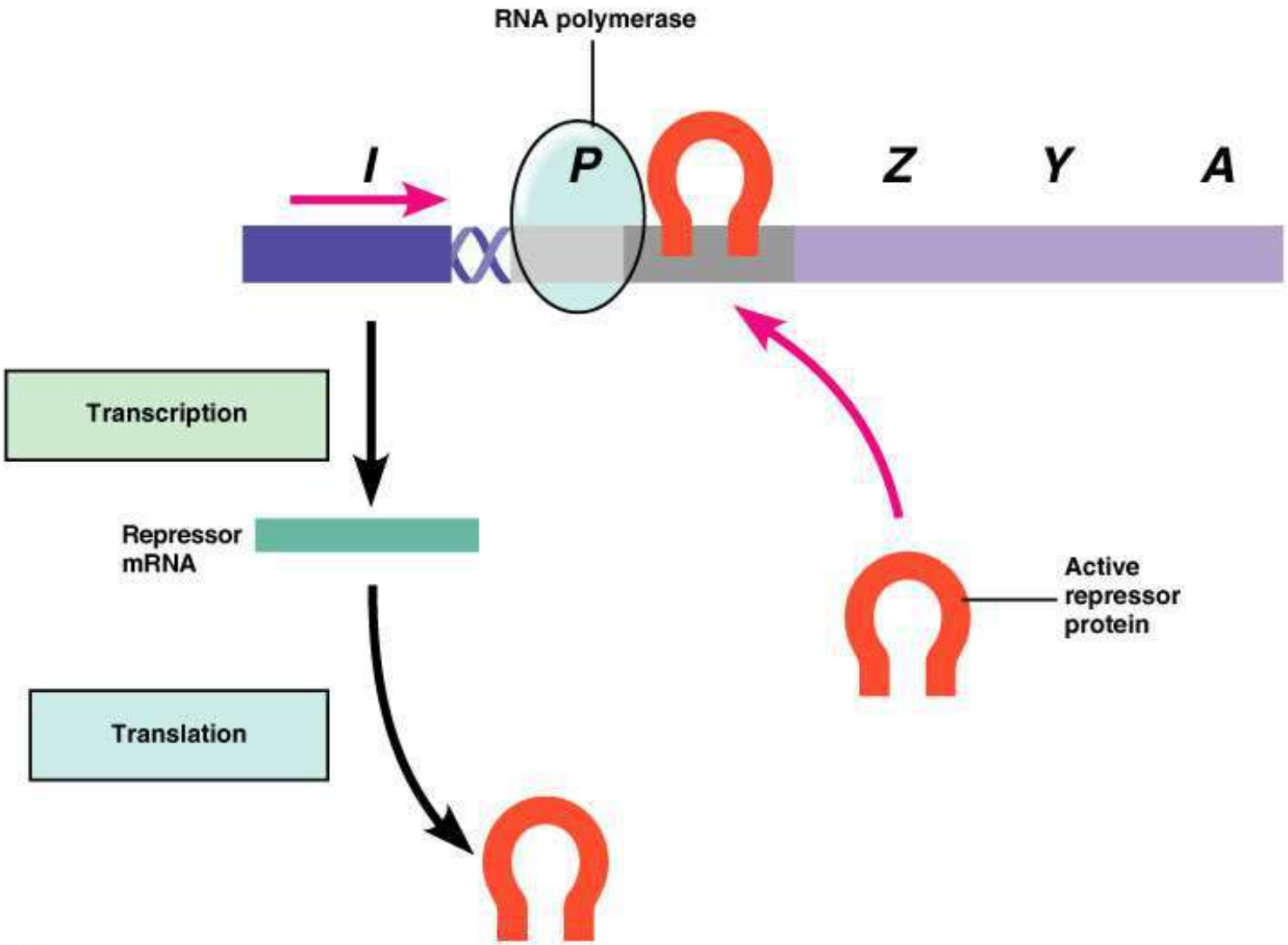
Operon



1 **Structure of the operon.** The operon consists of the promoter (*P*), and operator (*O*) sites, and structural genes which code for the protein. The operon is regulated by the product of the regulatory gene (*I*).

Figure 8.14.1

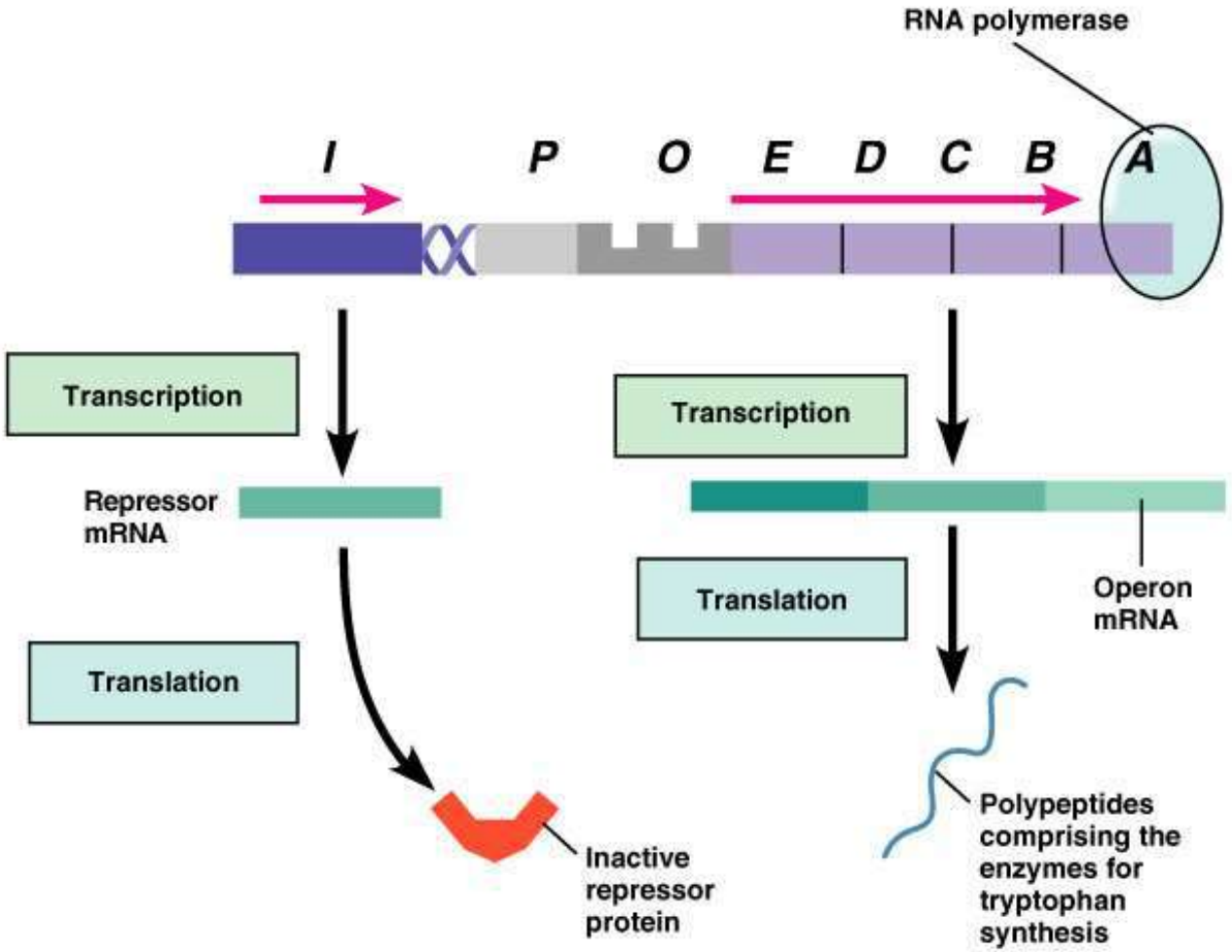
Regulation of Gene Expression



2 **Repressor active, operon off.** The repressor protein binds with the operator, preventing transcription from the operon.

Figure 8.14.2

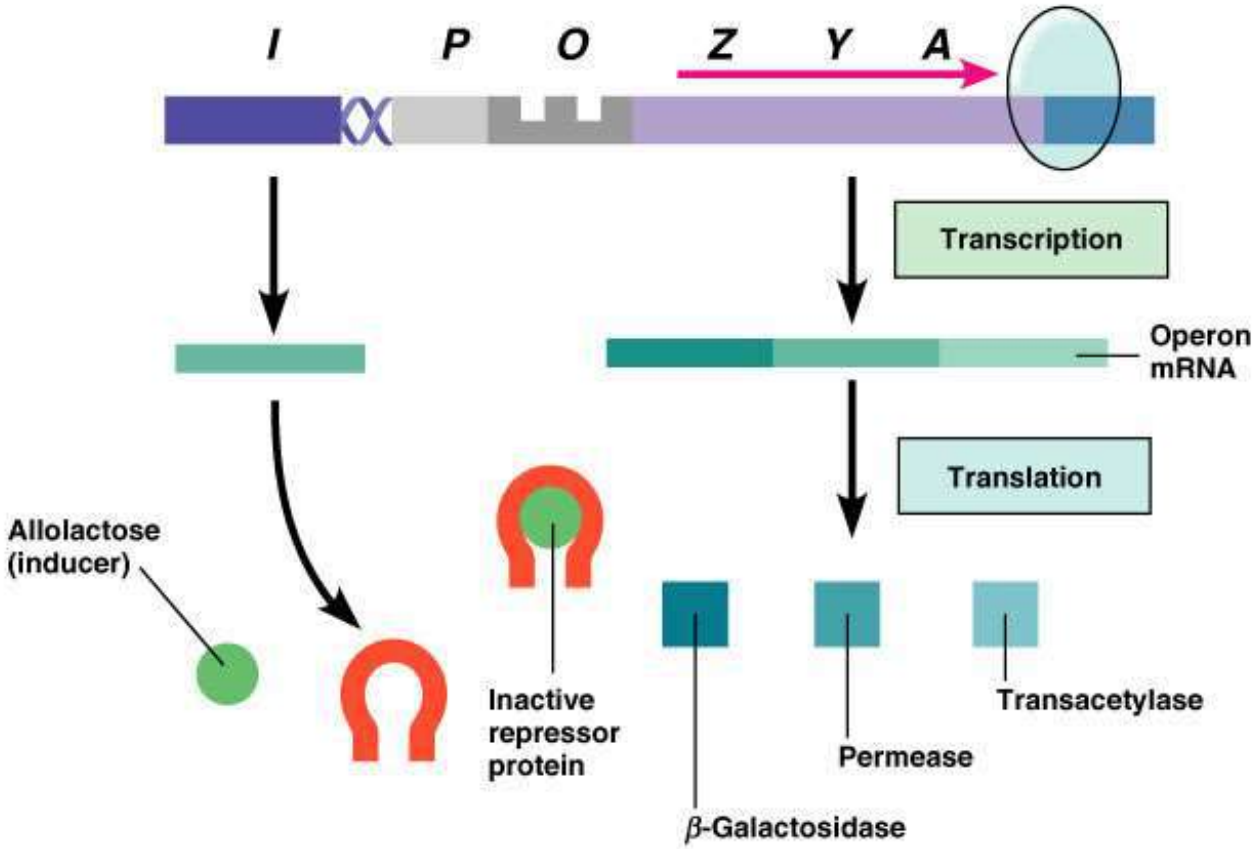
Regulation of Gene Expression



2 **Repressor inactive, operon on.** The repressor is inactive and transcription and translation proceed leading to the synthesis of tryptophan.

Figure 8.14.3

Regulation of Gene Expression

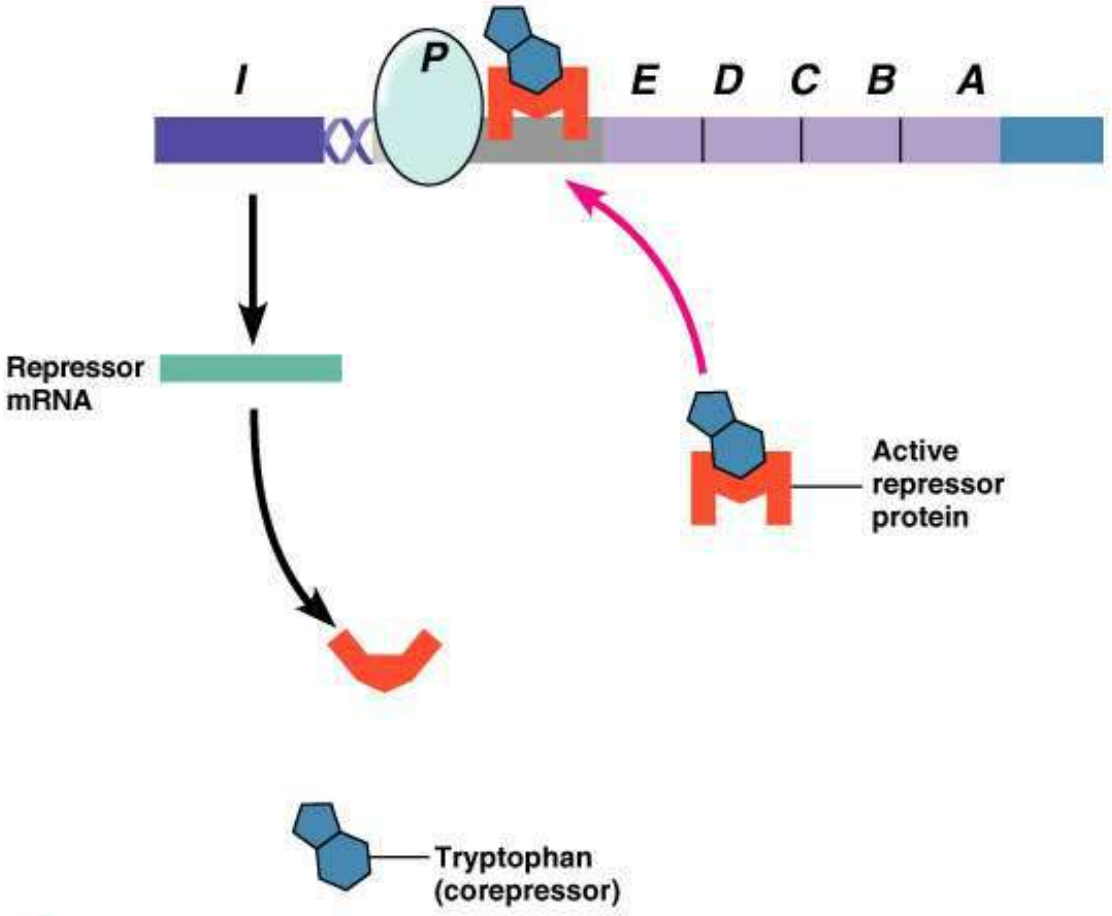


3 **Repressor inactive, operon on.** When the inducer allolactose binds to the repressor protein, the inactivated repressor can no longer block transcription. The structural genes are transcribed, ultimately resulting in the production of the enzymes needed for lactose catabolism.

(a) An inducible operon

Figure 8.14.4

Regulation of Gene Expression

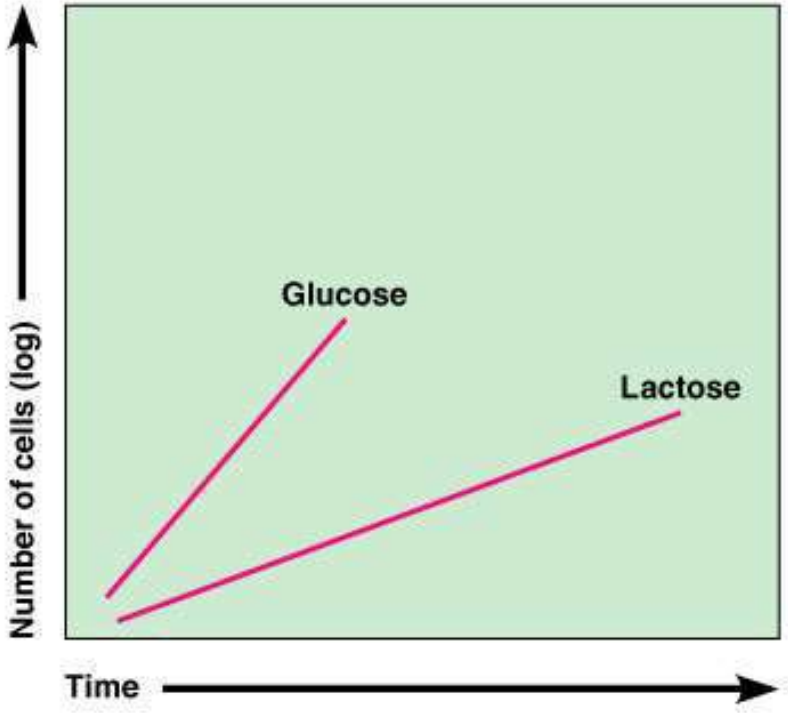


3 **Repressor active, operon off.** When the corepressor tryptophan binds to the repressor protein, the activated repressor binds with the operator, preventing transcription from the operon.

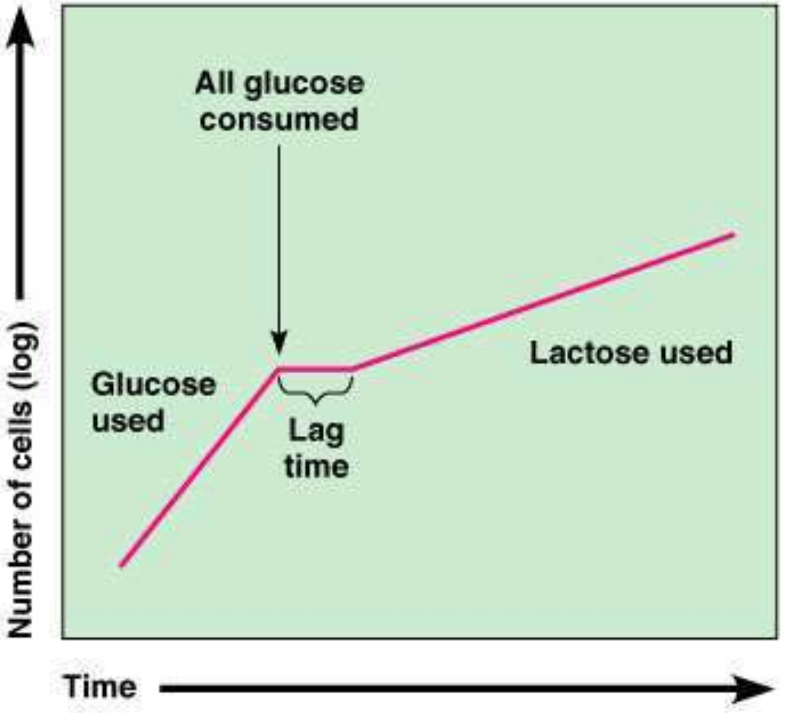
(b) A repressible operon

Figure 8.14.5

Regulation of Gene Expression



(a) Growth on glucose or lactose alone



(b) Growth on glucose and lactose combined

Figure 8.15

TORTORA • FUNKE • CASE

Microbiology

AN INTRODUCTION

EIGHTH EDITION

Classification of Medically Important Bacteria

Chapter 5

PowerPoint® Lecture Slide Presentation prepared by Christine L. Case

Taxonomy

- Taxonomy
  - The science of classifying organisms
  - Provides universal names for organisms
  - Provides a reference for identifying organisms

Taxonomy

- Systematics or phylogeny
  - The study of the evolutionary history of organisms
- All Species Inventory (2001-2025)
  - To identify all species of life on Earth

Taxonomy

- 1735 Plant and Animal Kingdoms
- 1857 Bacteria & fungi put in the Plant Kingdom
- 1866 Kingdom Protista proposed for bacteria, protozoa, algae, & fungi
- 1937 "Prokaryote" introduced for cells "without a nucleus"
- 1961 Prokaryote defined as cells in which nucleoplasm is not surrounded by a nuclear membrane
- 1959 Kingdom Fungi
- 1968 Kingdom Prokaryotae proposed
- 1978 Two types of prokaryotic cells found

The Three-Domain System

TABLE 10.1 Some Characteristics of Archaea, Bacteria, and Eukarya

	Archaea	Bacteria	Eukarya
	 *Methanosarcina*	 *E. coli*	 *Amoeba*
Cell Type	Prokaryotic	Prokaryotic	Eukaryotic
Cell Wall	Varies in composition; contains no peptidoglycan	Contains peptidoglycan	Varies in composition; contains carbohydrates
Membrane Lipids	Composed of branched carbon chains attached to glycerol by ether linkage	Composed of straight carbon chains attached to glycerol by ester linkage	Composed of straight carbon chains attached to glycerol by ester linkage
First Amino Acid in Protein Synthesis	Methionine	Formylmethionine	Methionine
Antibiotic Sensitivity	No	Yes	No
rRNA Loop*	Lacking	Present	Lacking
Common Arm of tRNA†	Lacking	Present	Present

*Binds to ribosomal protein; found in all bacteria.
†A sequence of bases in tRNA found in all eukaryotes and bacteria: guanine-thymine-pseudouridine-cytosine-guanine.

The Three-Domain System

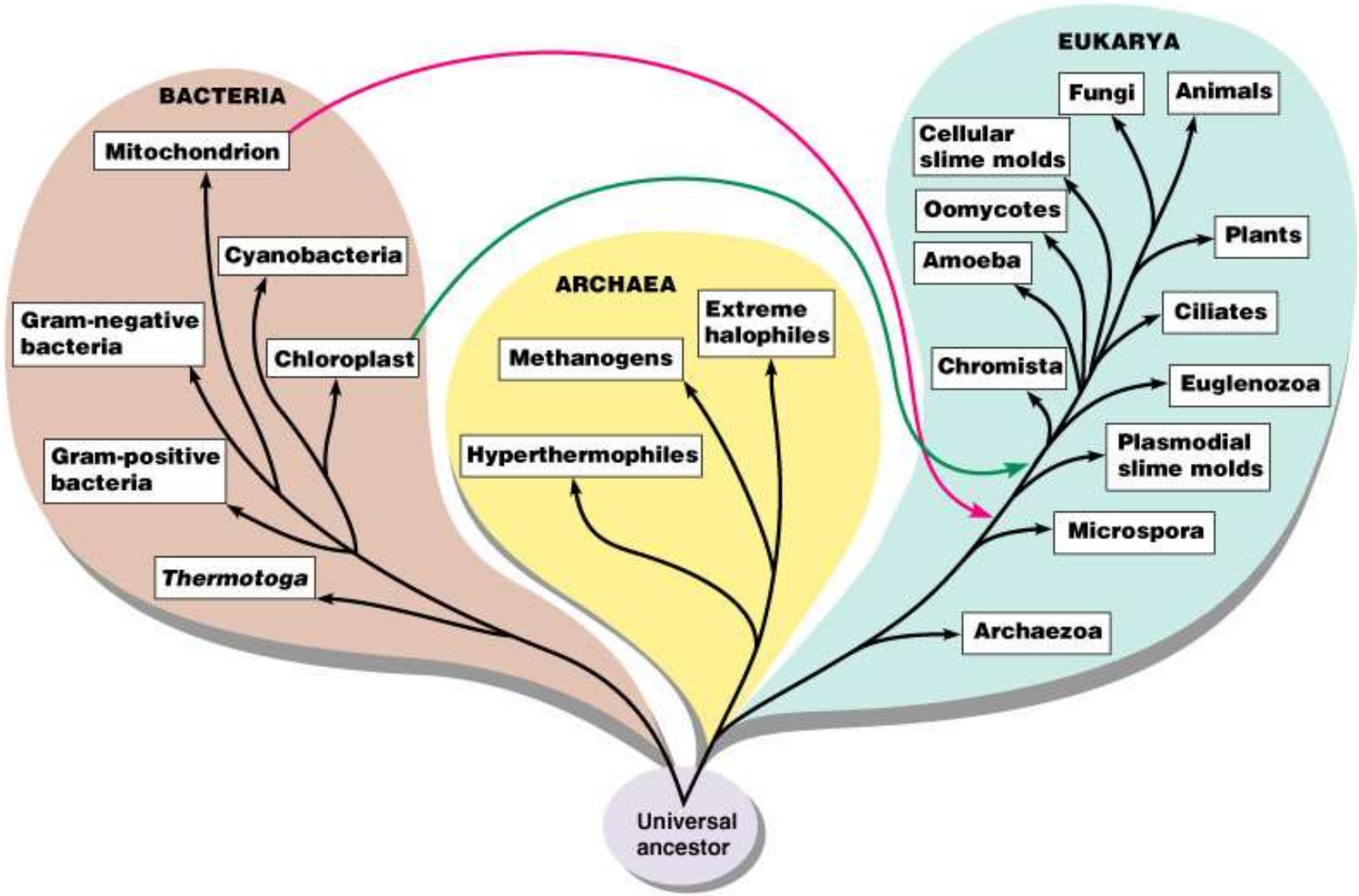
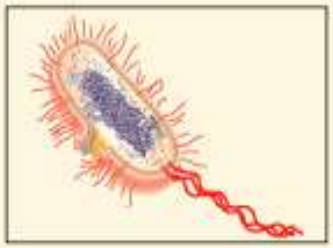
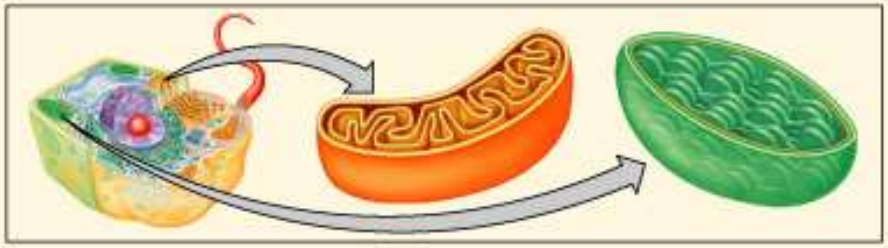


Figure 10.1

TABLE 10.2 **Prokaryotic Cells and Eukaryotic Organelles Compared**

	Prokaryotic Cell	Eukaryotic Cell	Eukaryotic Organelles (Mitochondria and Chloroplasts)
DNA	Circular	Linear	Circular
Histones	No	Yes	No
Ribosomes	70S	80S	70S
Growth	Binary fission	Mitosis	Binary fission





Endosymbiotic Theory

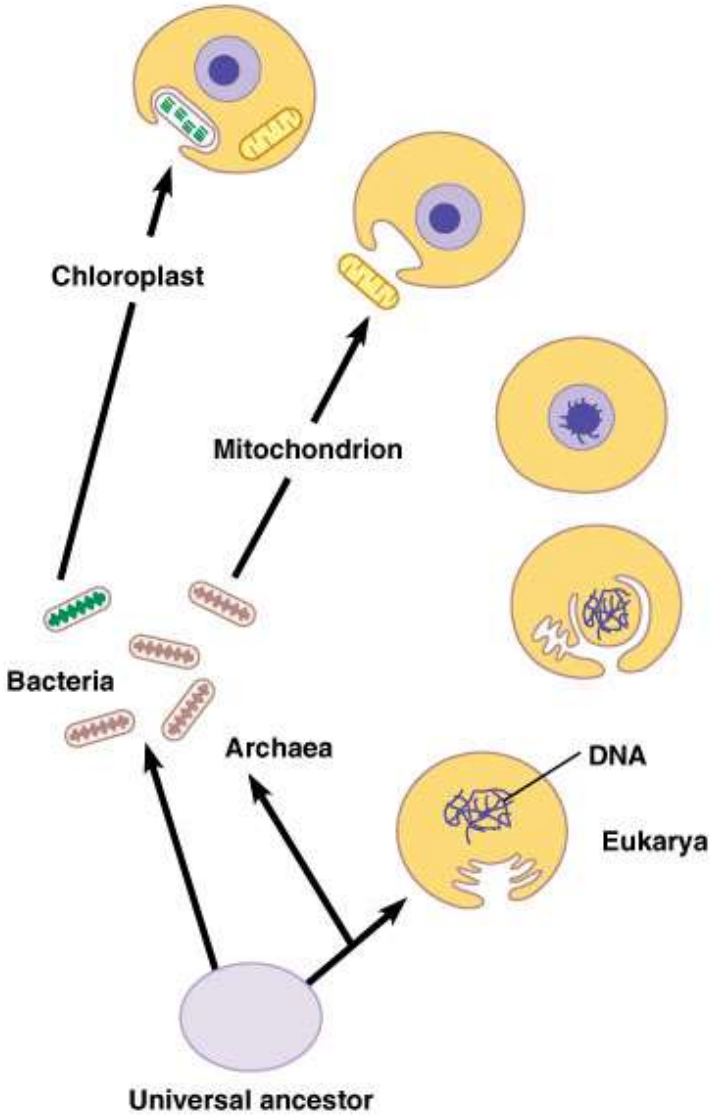


Figure 10.2

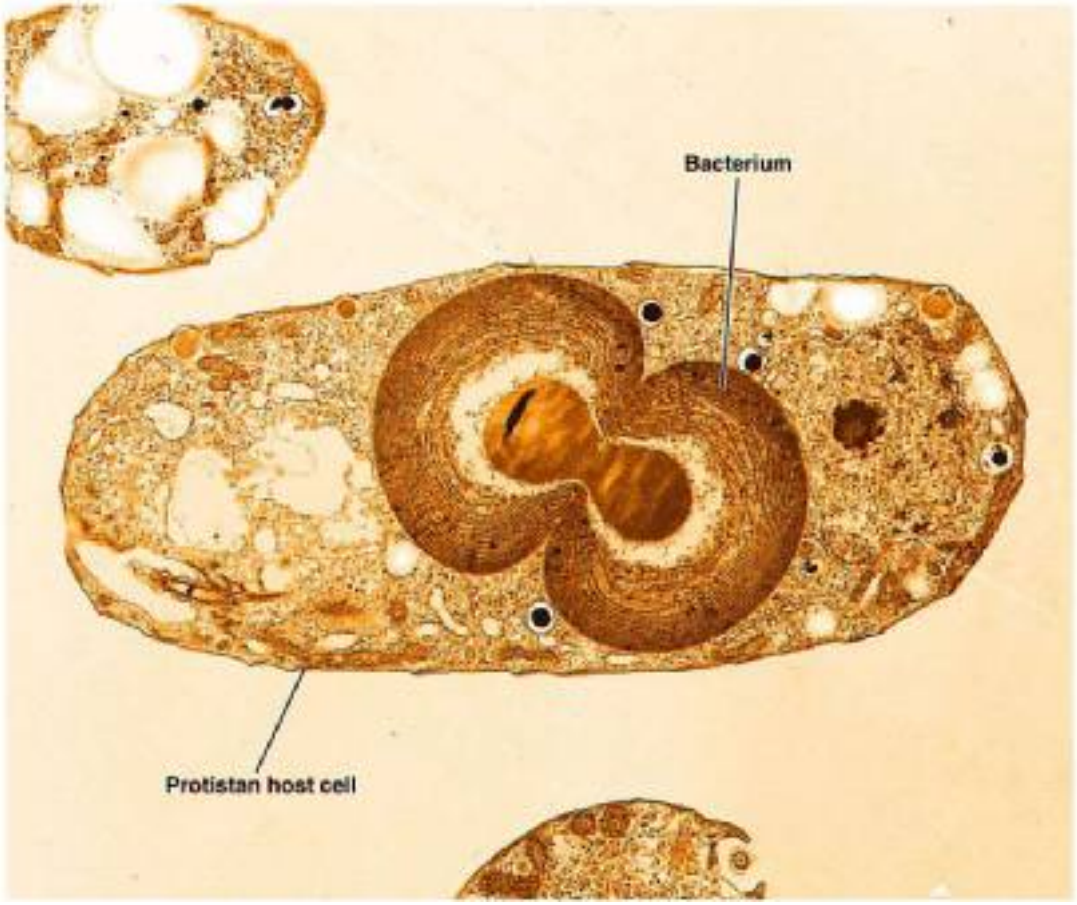


Figure 10.3

Scientific Names

Scientific binomial	Source of Genus name	Source of Specific epithet
Kbebsiella pneumoniae	Honors Edwin Klebs	The disease
Pfiesteria piscicida	Honors Lois Pfiester	Disease in fish
Salmonella typhimurium	Honors Daniel Salmon	Stupor (typh-) in mice (muri-)
Streptococcus pyogenes	Chains of cells (strepto-)	Forms pus (pyo-)
Penicillium notatum	Tuftlike (penicill-)	Spores spread in wind (nota)
Trypanosoma cruzi	Corkscrew-like (trypano-, borer; soma-body)	Honors Oswaldo Cruz

Scientific nomenclature

- The taxonomic hierarchy:
  - Domain
  - Kingdom
  - Phylum (or division for bacteria)
  - Class
  - Order
  - Family
  - Genus
  - Species

Taxonomic Hierarchy

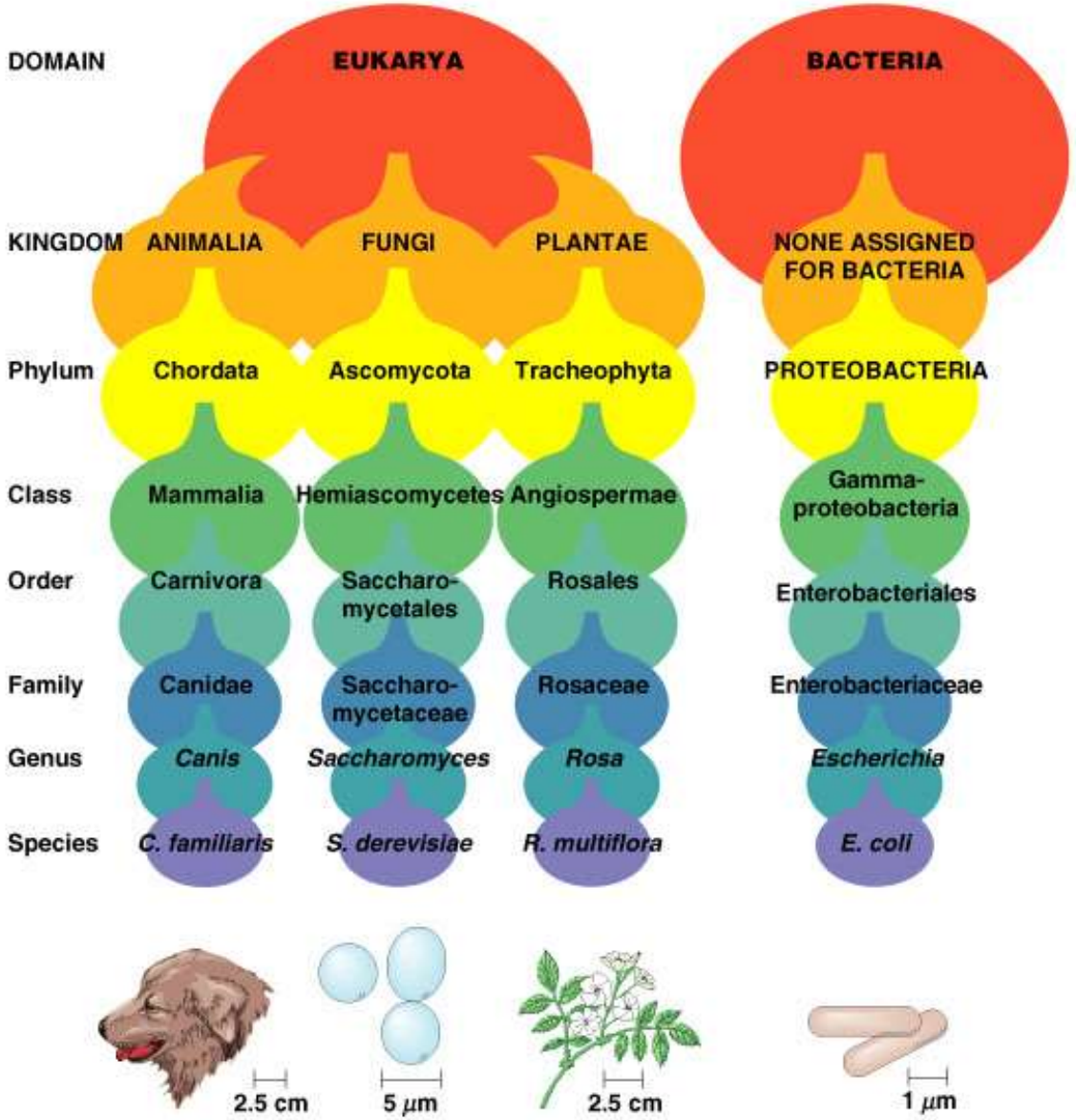


Figure 10.5

Species Definition

- Eukaryotic species:
  - A group of closely related organisms that breed among themselves
- Prokaryotic species:
  - A population of cells with similar characteristics
  - Clone: Population of cells derived from a single cell
  - Strain: Genetically different cells within a clone
- Viral species:
  - Population of viruses with similar characteristics that occupies a particular ecological niche

Domain Eukarya

- Animalia: Multicellular; no cell walls; chemoheterotrophic
- Plantae: Multicellular; cellulose cell walls; usually photoautotrophic
- Fungi: Chemoheterotrophic; unicellular or multicellular; cell walls of chitin; develop from spores or hyphal fragments
- Protista: A catchall for eukaryotic organisms that do not fit other kingdoms

Prokaryotes

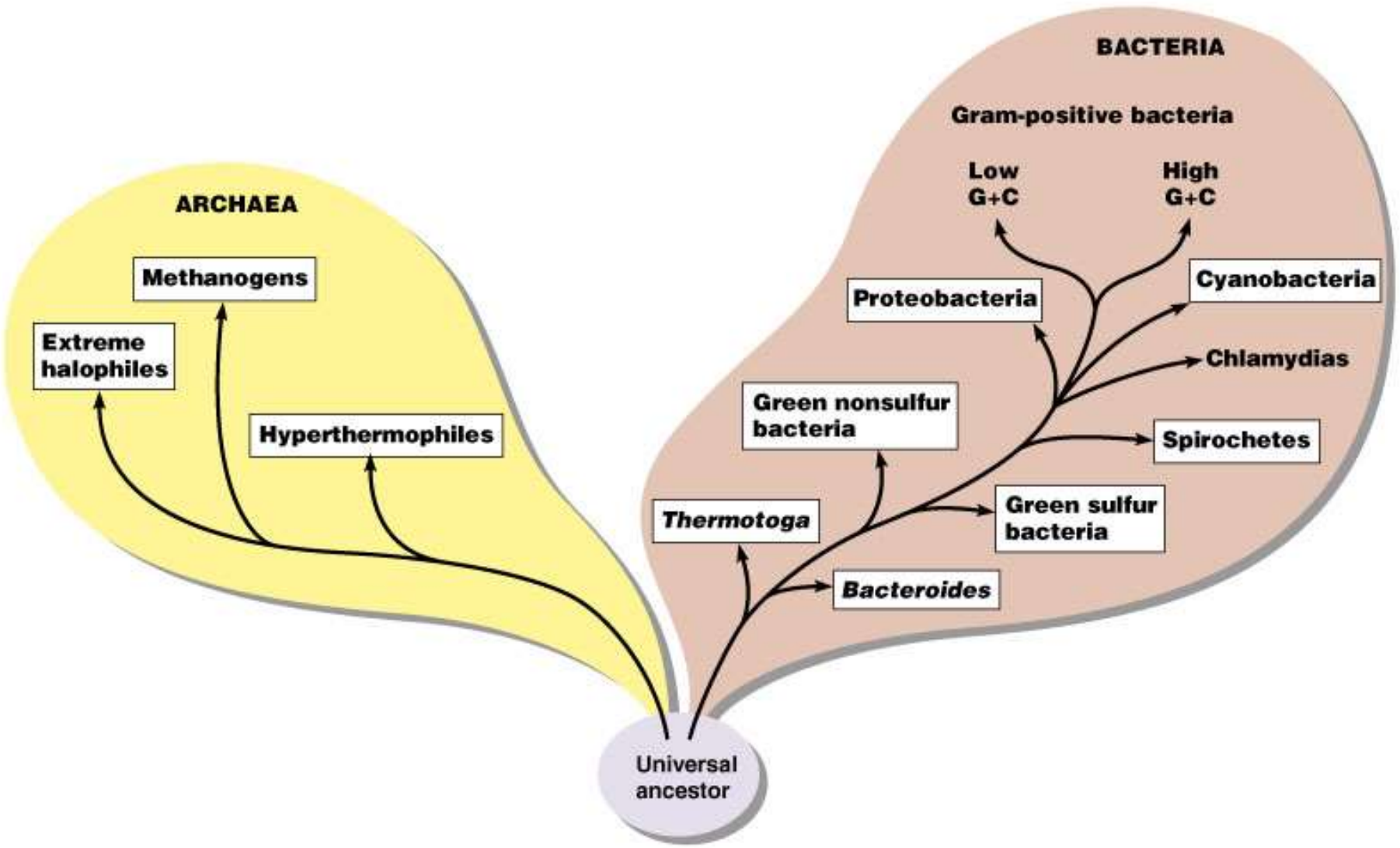


Figure 10.6

References

•• *Bergey's Manual of Determinative Bacteriology* •Provides identification schemes for identifying bacteria and archaea	•Morphology, differential staining, biochemical tests
•• *Bergey's Manual of Systematic Bacteriology* •Provides phylogenetic information on bacteria and archaea	•Based on rRNA sequencing
•• *Approved Lists of Bacterial Names* •Lists species of known prokaryotes	•Based on published articles

Identification Methods

- Morphological characteristics: Useful for identifying eukaryotes
- Differential staining: Gram staining, acid-fast staining
- Biochemical tests: Determines presence of bacterial enzymes

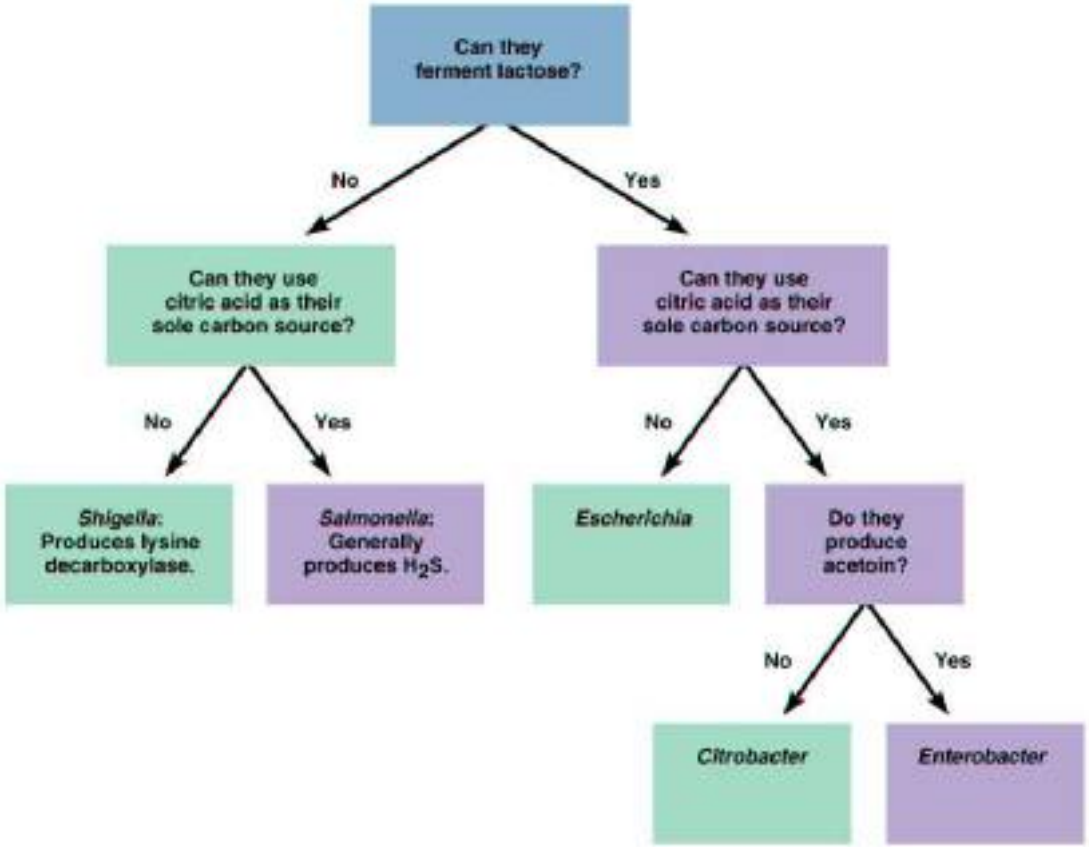
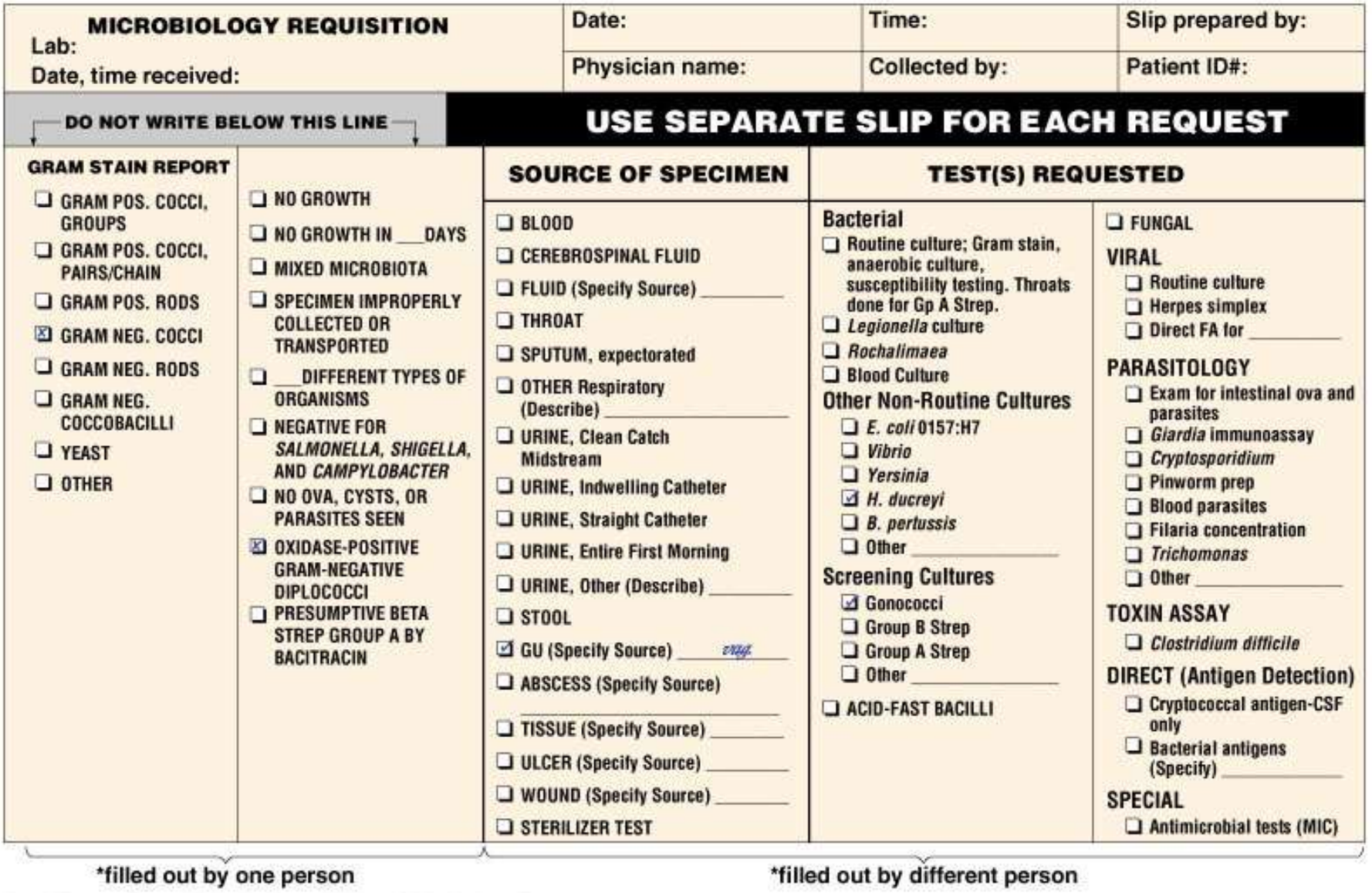


Figure 10.8



MICROBIOLOGY REQUISITION

Lab:

Date, time received:

Date:

Time:

Slip prepared by:

Physician name:

Collected by:

Patient ID#:

DO NOT WRITE BELOW THIS LINE

USE SEPARATE SLIP FOR EACH REQUEST

GRAM STAIN REPORT

- ☐ GRAM POS. COCCI, GROUPS
- ☐ GRAM POS. COCCI, PAIRS/CHAIN
- ☐ GRAM POS. RODS
- ☒ GRAM NEG. COCCI
- ☐ GRAM NEG. RODS
- ☐ GRAM NEG. COCCOBACILLI
- ☐ YEAST
- ☐ OTHER

- ☐ NO GROWTH
- ☐ NO GROWTH IN ___DAYS
- ☐ MIXED MICROBIOTA
- ☐ SPECIMEN IMPROPERLY COLLECTED OR TRANSPORTED
- ☐ ___DIFFERENT TYPES OF ORGANISMS
- ☐ NEGATIVE FOR *SALMONELLA, SHIGELLA,* AND *CAMPYLOBACTER*
- ☐ NO OVA, CYSTS, OR PARASITES SEEN
- ☒ OXIDASE-POSITIVE GRAM-NEGATIVE DIPLOCOCCI
- ☐ PRESUMPTIVE BETA STREP GROUP A BY BACITRACIN

SOURCE OF SPECIMEN

- ☐ BLOOD
- ☐ CEREBROSPINAL FLUID
- ☐ FLUID (Specify Source) _____
- ☐ THROAT
- ☐ SPUTUM, expectorated
- ☐ OTHER Respiratory (Describe) _____
- ☐ URINE, Clean Catch Midstream
- ☐ URINE, Indwelling Catheter
- ☐ URINE, Straight Catheter
- ☐ URINE, Entire First Morning
- ☐ URINE, Other (Describe) _____
- ☐ STOOL
- ☑ GU (Specify Source) ___vag___
- ☐ ABSCESS (Specify Source) _____
- ☐ TISSUE (Specify Source) _____
- ☐ ULCER (Specify Source) _____
- ☐ WOUND (Specify Source) _____
- ☐ STERILIZER TEST

TEST(S) REQUESTED

Bacterial

- ☐ Routine culture; Gram stain, anaerobic culture, susceptibility testing. Throats done for Gp A Strep.
- ☐ *Legionella* culture
- ☐ *Rochalimaea*
- ☐ Blood Culture

Other Non-Routine Cultures

- ☐ *E. coli* 0157:H7
- ☐ *Vibrio*
- ☐ *Yersinia*
- ☑ *H. ducreyi*
- ☐ *B. pertussis*
- ☐ Other _____

Screening Cultures

- ☑ Gonococci
- ☐ Group B Strep
- ☐ Group A Strep
- ☐ Other _____

- ☐ ACID-FAST BACILLI

- ☐ FUNGAL

VIRAL

- ☐ Routine culture
- ☐ Herpes simplex
- ☐ Direct FA for _____

PARASITOLOGY

- ☐ Exam for intestinal ova and parasites
- ☐ *Giardia* immunoassay
- ☐ *Cryptosporidium*
- ☐ Pinworm prep
- ☐ Blood parasites
- ☐ Filaria concentration
- ☐ *Trichomonas*
- ☐ Other _____

TOXIN ASSAY

- ☐ *Clostridium difficile*

DIRECT (Antigen Detection)

- ☐ Cryptococcal antigen-CSF only
- ☐ Bacterial antigens (Specify) _____

SPECIAL

- ☐ Antimicrobial tests (MIC)

*filled out by one person

*filled out by different person

Figure 10.7

Chapter 6

The Human Microbiome

The Microbiome (Normal Flora)

- **General aspects**
  - Remember definition: *organisms frequently found on or within body of healthy individuals*
  - Most are bacteria, but some are viruses, fungi, and protozoa
    - We do not carry all of them all of the time
    - Each person has individualized normal flora

The Microbiome (Normal Flora)

- Some are found only on body; others also found in environment
- Problem: some people have transient normal flora (pathogens)
  - Example: about 10% of population have meningococcus or pneumococcus as normal flora

Importance

- **Opportunistic infections:** normal flora in unusual sites; for example:
  - *Bacteroides* from intestine into deeper tissues as a result of trauma (or surgery)
  - Staphylococci from skin and nose
  - Streptococci and Gram⁻ cocci from throat and mouth
  - ❖ Carrier state: lead to colonization
  - ❖ Dysbioses: any change in the microbiome composition (lead to diseases as obesity & IBD)

Importance

- Depends on pathogen *and* on defenses of host:
  - *Candida* (yeast) causes pneumonia in people undergoing cancer chemotherapy
  - *Pneumocystis carinii* (common inhabitant of lung) causes pneumonia and death in AIDS patients

Ecology of microbiota

- **Sterile sites**—pathogens in these *definitely* indicate disease
  - Blood
  - Cerebrospinal fluid
  - Synovial fluid
  - Deep tissues

Contribution of microbiome to health and disease

- **1. Immune system:** Antigenic stimulation by normal flora— do not have high antibody titers
  - Serve as defense mechanism even in low concentration
  - Bacterial stimulation leads to production of IgA that is secreted through mucus membranes
    - Probably interfere with colonization of deeper tissues

Contribution of microbiome to health and disease

- Cross-reactivity does not normally cause disease
  - Possible for antibodies cross-reactive to microbial Ag to cause problem
    - Lupus erythematosus—production of Ab against host DNA
      - Some evidence that Ag may be cross-reacting bacterial LPS
    - May cross-react with pathogen (meningococcus)
    - **Germ-free animal model**

Contribution of microbiome to health and disease

- **2. Colonization resistance**
  - Confer resistance or susceptibility to pathogen colonization
    - HUS caused by EHEC (O157:H7)
    - After antibiotics….*C. difficille* can cause PMC

Contribution of microbiome to health and disease

- **3. Contribution to nutrition and health:**
  - Digest plant fibers (long chains), aid in absorption.
  - Synthesis of B vitamins and K
  - Aid in the absorption of minerals as iron

Physical & Chemical Aspects

- **Antibiotic effects:** wipes out normal flora
  - Both endogenous and exogenous organisms can cause disease
    - Infecting dose of *Salmonella* decreases one million-fold when mice given streptomycin
    - Patients treated with some potent antibiotics:
      - Suffer from diarrhea due to overgrowth of yeasts, and staphylococci
      - Administration of clindmycin-*Clostridium difficile* (minor member of normal flora) causes pseudomembranous colitis

Physical & Chemical Aspects

- **Source of carcinogens**
  - Large intestinal flora
    - Many potential carcinogens are only active after being modified
      - Some modifications are carried out by enzymes of intestinal bacteria; example: cyclamate converted to bladder carcinogen (cycloheximide) by bacterial sulfatases.
    - Importance of carcinogen production not clear

Microbiome of the GI tract

- 20% of feces is anaerobes (1011/g)
- Firmicutes (64%), Bacteroidetes (23%)
- Rest are Proteobacteria & Actinobacteria
- Experiments on mice's to proof the role of microbiome on obesity.
- Role of microbiome in IBD

Microbiome of the skin

- Mainly *S. epidermidis* (1011/g), less *S. aureus*
- *Anaerobes as Peptostreptococcus acnes*
- *Candida albicans!*

Microbiome of the R.T.

- Nose: mainly *S. aureus*
- Throat contains: viridans streptococci, *Neisseria* species, and *S. epidermidis*
- Mouth: viridans streptococci (*S. mutans*)
- Gingiva: mainly anaerobes as Bacteroides, Prevotella, Fusobacterium, Clostridia, Peptostreptococcus & *Actinomyces israelii*

Microbiome of the genitourinary tract

- Lactobacilli (puberty/childhood/menopause/estrogen)
- *C. albicans! (antibiotics may lead to vaginitis)*
- *May colonized with E. coli (20%) or GBS*
- *Also S. aureus (5%) (TSS),*
- *Area around the urethra contains M. smegmatis, and skin contains S. saprophyticus*

TORTORA • FUNKE • CASE

Microbiology

AN INTRODUCTION

EIGHTH EDITION

Pathogenesis

PowerPoint® Lecture Slide Presentation prepared by Christine L. Case

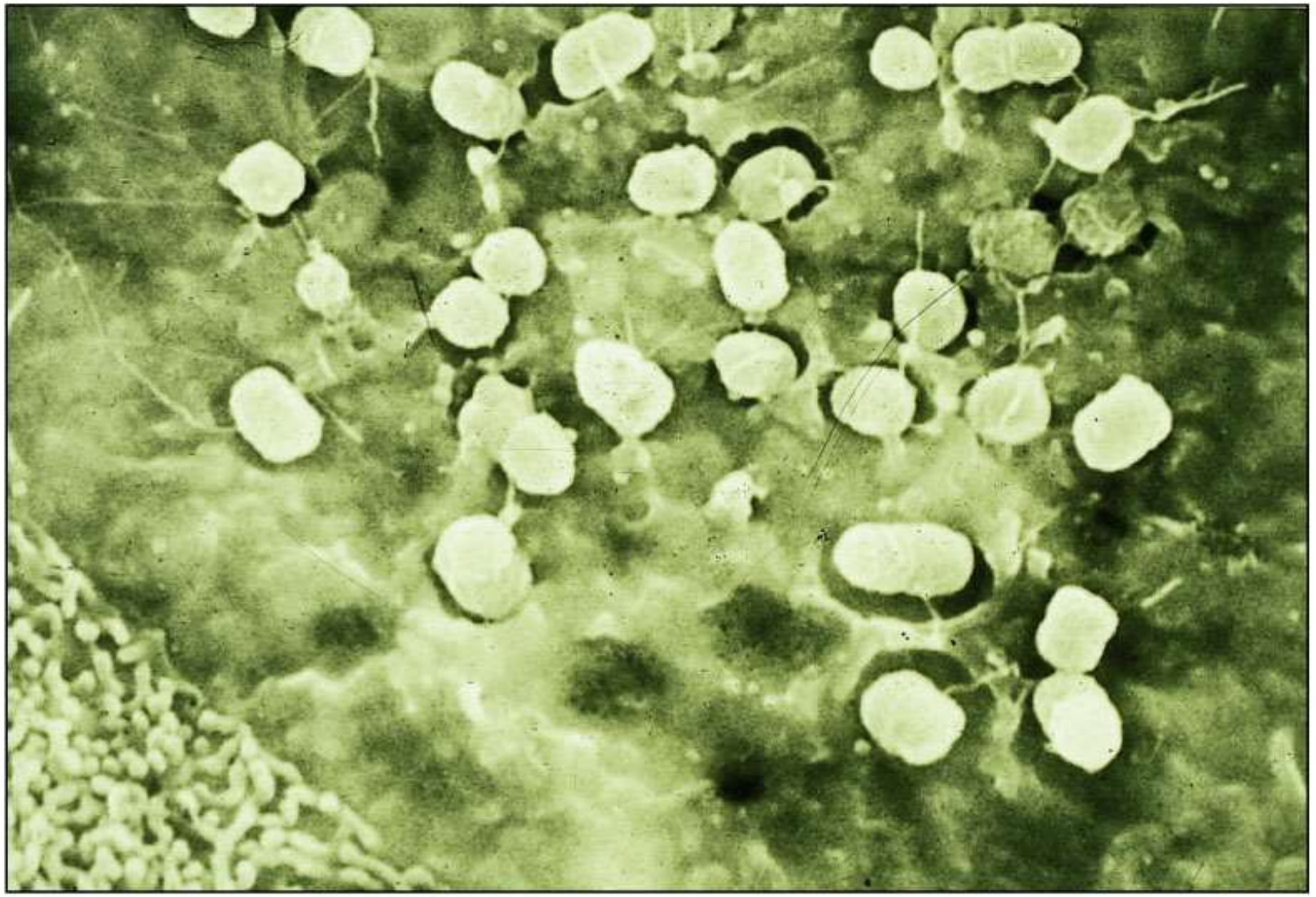
Microbial Mechanisms of Pathogenicity

- Pathogenicity: The ability to cause disease
- Virulence: The extent of pathogenicity or "quantitative measure of pathogenicity"
- Virulence factots:
- Obligate intracellular parasites (*Chlamydia, Rickettsia)*
- *Why do people get infectious diseases?*

Portals of Entry

- Mucous membranes:
  - * Inhalation (via the respiratory tract)
  - * Absorption (via mucous membranes as the eyes)
  - * Ingestion (via the gastrointestinal tract)
  - * Urogenital tract
- Skin:
  - * Inoculation (as the result of an inoculation injury)
- Parenteral route: (needles, indwelling catheters)

Penetration into the Host Cell



Numbers of Invading Microbes

- **ID_{50}:** Infectious dose for 50% of the test population
- **LD_{50}:** Lethal dose (of a toxin) for 50% of the test population

Types of Bacterial Infections

- Toxin production
- Invasion and Inflammation
- Many of infections are communicable
- Epidemic, pandemic, endemic infection
- Subclinical, latent, chronic infection

Stages of Bacterial Pathogenesis

- Transmission
- Evasion
- Adherence
- Colonization
- Disease symptoms
- Host responses
- Progression or resolution

Transmission

- Direct
- Indirect
- Vector (zoonoses)
- Vertical transmission
- Horizontal Transmission

Adherence to cell surfaces

- Adhesions/ligands bind to receptors on host cells
  - Glycocalyx — *Streptococcus mutans*
  - Fimbriae — *Escherichia coli*
  - M protein — *Streptococcus pyogenes*
  - Opa protein — *Neisseria gonorrhoeae*
  - Tapered end — *Treponema pallidum*

TABLE 7–8 Surface Virulence Factors Important for Bacterial Pathogenesis

Organism	Virulence Factor	Used in Vaccine	Comments
Gram-positive cocci			
Streptococcus pneumoniae	Polysaccharide capsule	Yes	Determines serotype
Streptococcus pyogenes	M protein	No	Determines serotype[1]
Staphylococcus aureus	Protein A	No	Binds to Fc region of IgG, which prevents activation of complement
Gram-negative cocci			
Neisseria meningitides	Polysaccharide capsule	Yes	Determines serotype
Gram-positive rods			
Bacillus anthracis	Polypeptide capsule	No	
Gram-negative rods			
Haemophilus influenzae	Polysaccharide capsule	Yes	Determines serotype
Klebsiella pneumoniae	Polysaccharide capsule	No	
Escherichia coli	Protein pili	No	Causes adherence
Salmonella typhi	Polysaccharide capsule	Yes	Not important for other salmonellae
Yersinia pestis	V and W proteins	No	

[1]Do not confuse the serotype with the grouping of streptococci, which is determined by the polysaccharide in the cell wall.

Invasion, Inflammation, & Intracellular survival

- Enzymes
- Antiphagocytic factots (capsule, cell wall protein as M protein of *S. pyogenes* & protein A of *S. aureus*)
- Pyogenic inflammation
- Granulomatous inflammation
- Intracellular survival (*Mycobacterium, Listeria, Legionella, Brucella*)
- *L. Monocytogenes* (actin rockets)
- Yersinia „Yops“ inhibit phagocytosis & TNF production
- Pathogenecity islands
- Pseudomembranes (diphtheria, PMC)

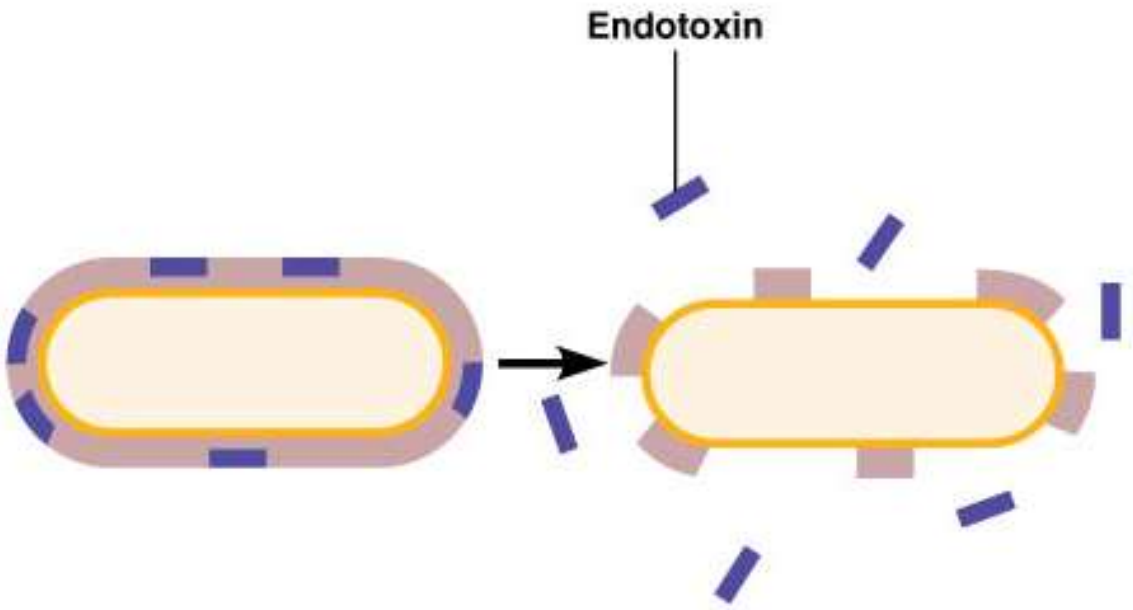
Enzymes

• Coagulase	Coagulate blood
• Kinases	Digest fibrin clots
• Hyaluronidase	Hydrolyses hyaluronic acid
• Collagenase	Hydrolyzes collagen
• IgA proteases	Destroy IgA antibodies
• Siderophores	Take iron from host iron-binding proteins
• Antigenic variation	Alter surface proteins

Toxins

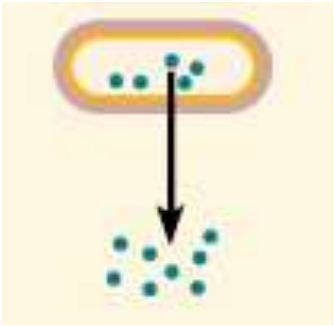
- **Toxin:** Substances that contribute to pathogenicity
- **Toxigenicity:** Ability to produce a toxin
- **Toxemia:** Presence of toxin the host's blood
- **Toxoid:** Inactivated toxin used in a vaccine
- **Antitoxin:** Antibodies against a specific toxin

Endotoxin



(b) Endotoxins are part of the outer portion of the cell wall (lipid A; see Figure 4.12c) of gram-negative bacteria. They are liberated when the bacteria die and the cell wall breaks apart.

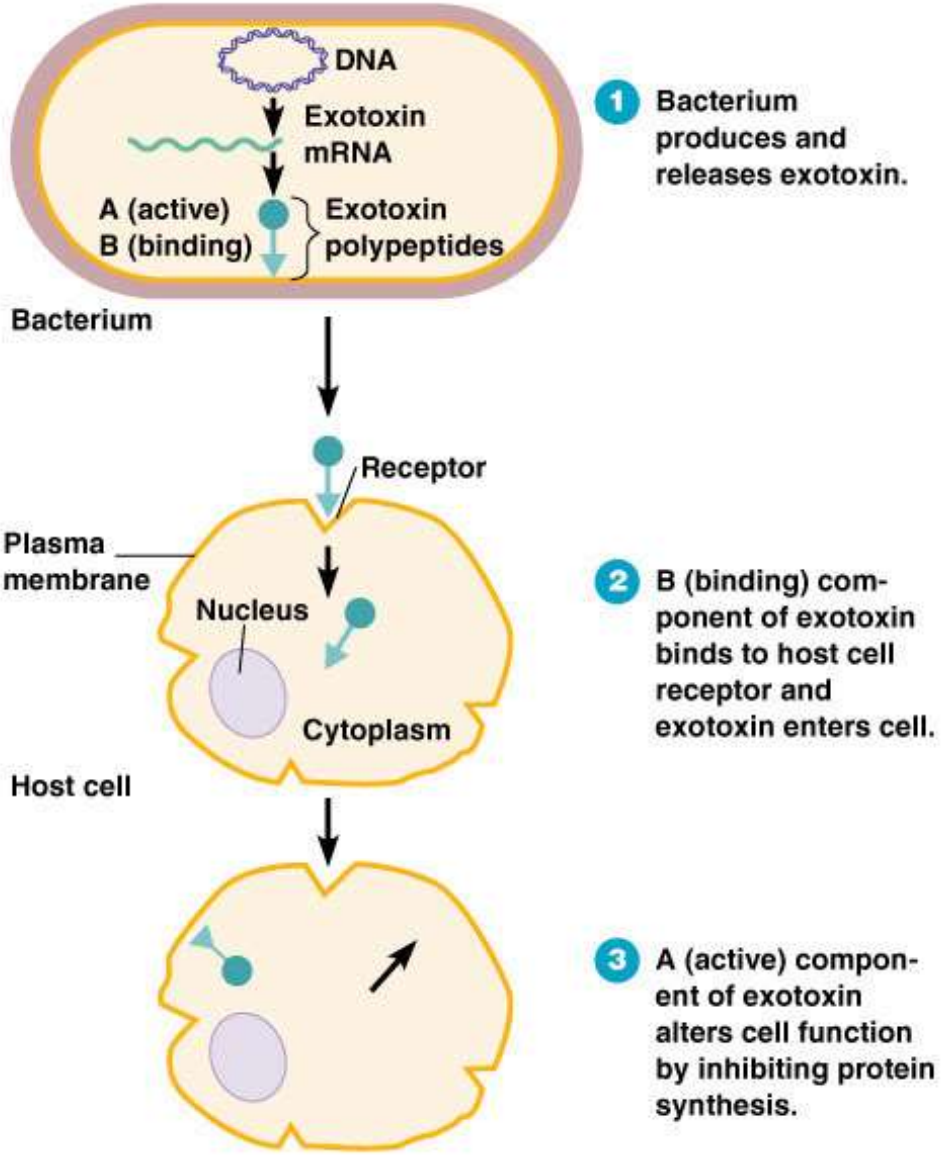
Exotoxin

	
Source	Mostly Gram +
Metabolic product	By-products of growing cell
Chemistry	Protein
Fever?	No
Neutralized by antitoxin	Yes
LD_{50}	Small

Exotoxins

- A-B toxins or type III toxins

(Injectosome)



Exotoxins

- Superantigens or type I toxins
  - Cause an intense immune response due to release of cytokines from host cells
  - Fever, nausea, vomiting, diarrhea, shock, death

Exotoxins

- Membrane-disrupting toxins or type II toxins
  - Lyse host's cells by:
    - Making protein channels in the plasma membrane (e.g., leukocidins, hemolysins)
    - Disrupting phospholipid bilayer

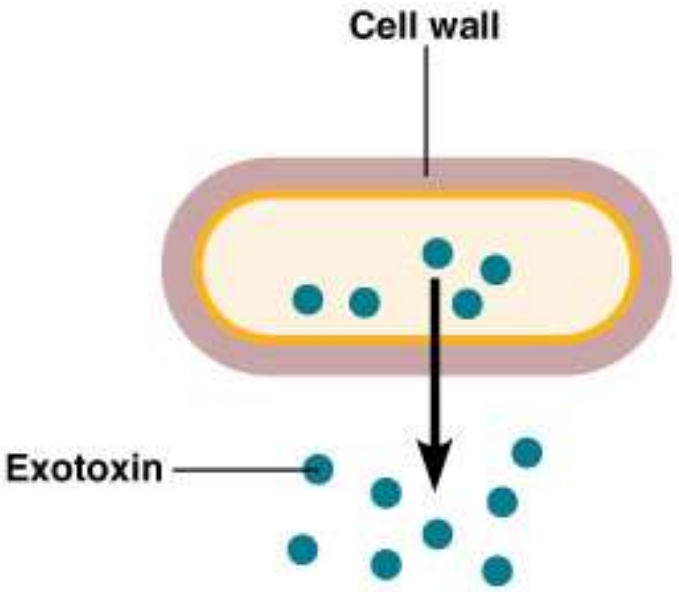
Exotoxins

	Exotoxin	Lysogenic conversion
• *Corynebacterium diphtheriae*	A-B toxin. Inhibits protein synthesis.	+
• *Streptococcus pyogenes*	Membrane-disrupting. Erythrogenic.	+
• *Clostridium botulinum*	A-B toxin. Neurotoxin	+
• *C. tetani*	A-B toxin. Neurotoxin	
• *Vibrio cholerae*	A-B toxin. Enterotoxin	+
• *Staphylococcus aureus*	Superantigen. Enterotoxin.	

Exotoxins

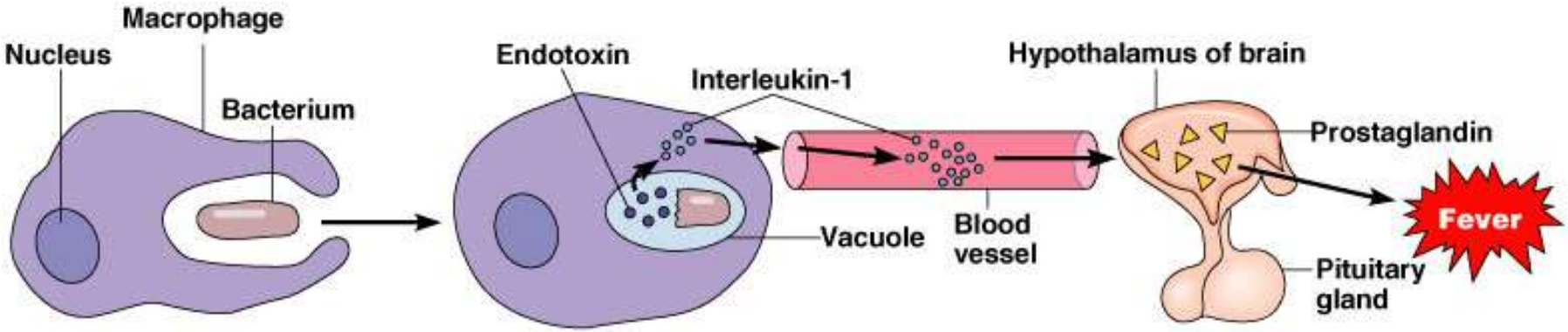
- A subunit of several exotoxin acts by ADP-ribosylation
- *Pseudomonas* exotoxin A & diphtheria toxin ADP-ribosylate elongation factor-2
- Cholera & *E. coli* toxins ADP-ribosylate G_s protein
- Pertussis toxin ADP-ribosylates G_i protein & inactivates it

Exotoxins

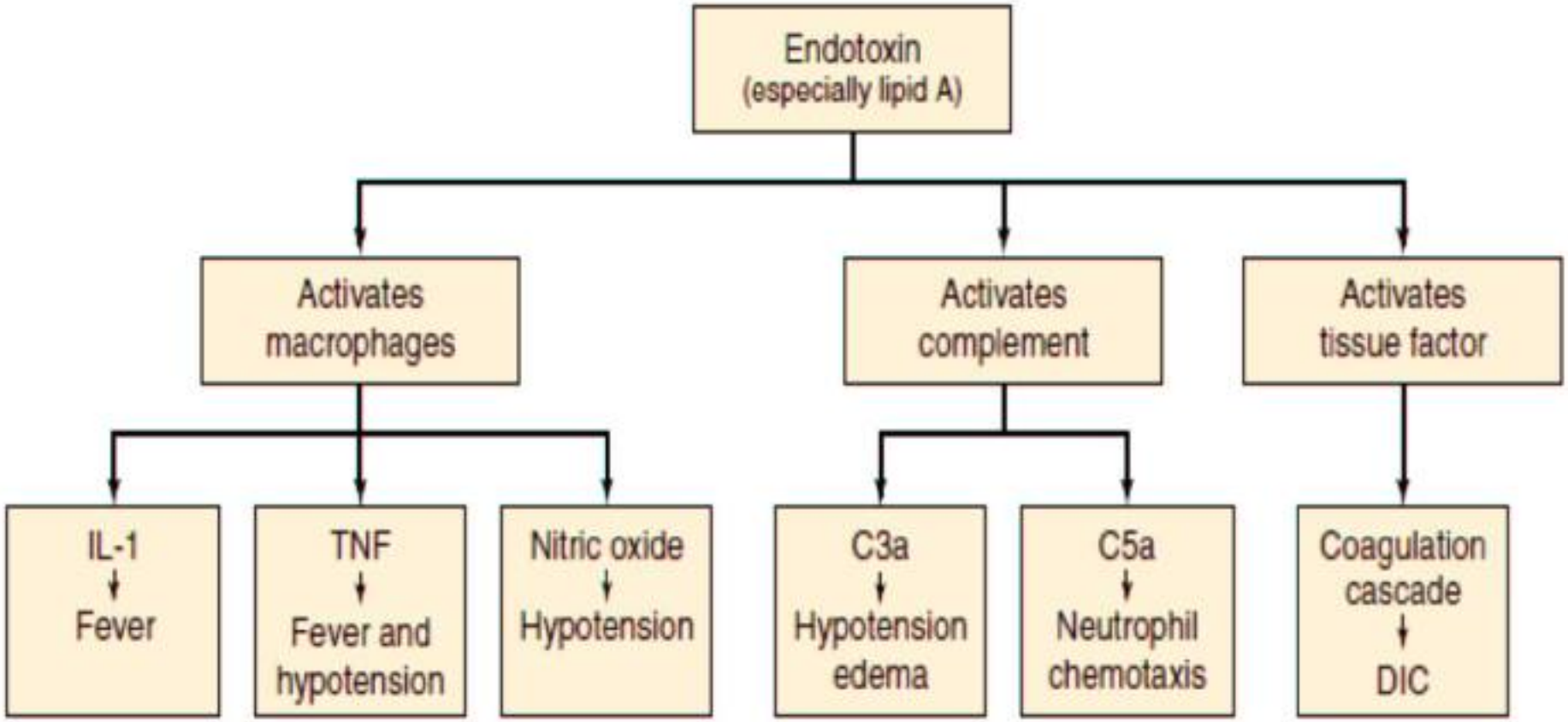


(a) **Exotoxins** are produced inside mostly gram-positive bacteria as part of their growth and metabolism. They are then secreted or released following lysis into the surrounding medium.

Endotoxins

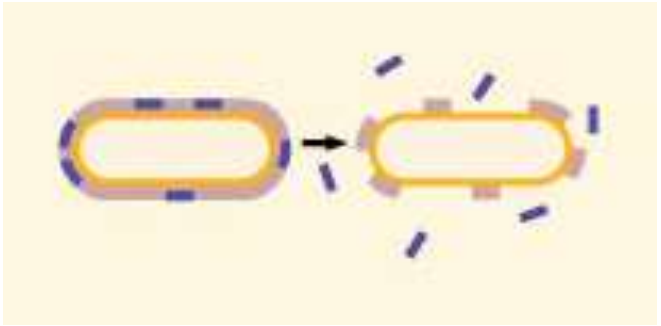


1. A macrophage ingests a gram-negative bacterium
2. The bacterium is degraded in a vacuole, releasing endotoxins that induce the macrophage to produce interleukin-1(IL-1)
3. IL-1 is released by the macrophage into the bloodstream, through which it travels to the hypothalamus of the brain
4. IL-1 induces the hypothalamus to produce prostaglandins, which reset the body's "thermostat" to a higher temperature, producing fever



Mode of action of Endotoxin

Endotoxins

	
Source	Gram–
Metabolic product	Present in LPS of outer membrane
Chemistry	Lipid
Fever?	Yes
Neutralized by antitoxin	No
LD_{50}	Relatively large

Gram positive Exotoxins

- Diphtheria toxin (ADP-ribosylation of EF-2)
- Tetanus toxin (prevents release of the inhibitory neurotransmitter glycine)
- Botulinum toxin (blocks the release of acetylcholine)
- *C. difficile* exotoxins (A & B)
- *C. perfringens* exotoxin (α-toxin, DNase, enterotoxin)
- *B. anthracis* (3 exotoxins): edema factor, adenylate cyclas, cyclic AMP; lethal factor, protease; protective Ag, formes pores
- TSST of *S. aureus* & some *S. pyogenes*
- Staphylococcal enterotoxins
- Exfoliatin of *S. aureus* (scalded skin syndrome)
- Erythrogenic toxin, *S. pyogenes* „scarlet fever“

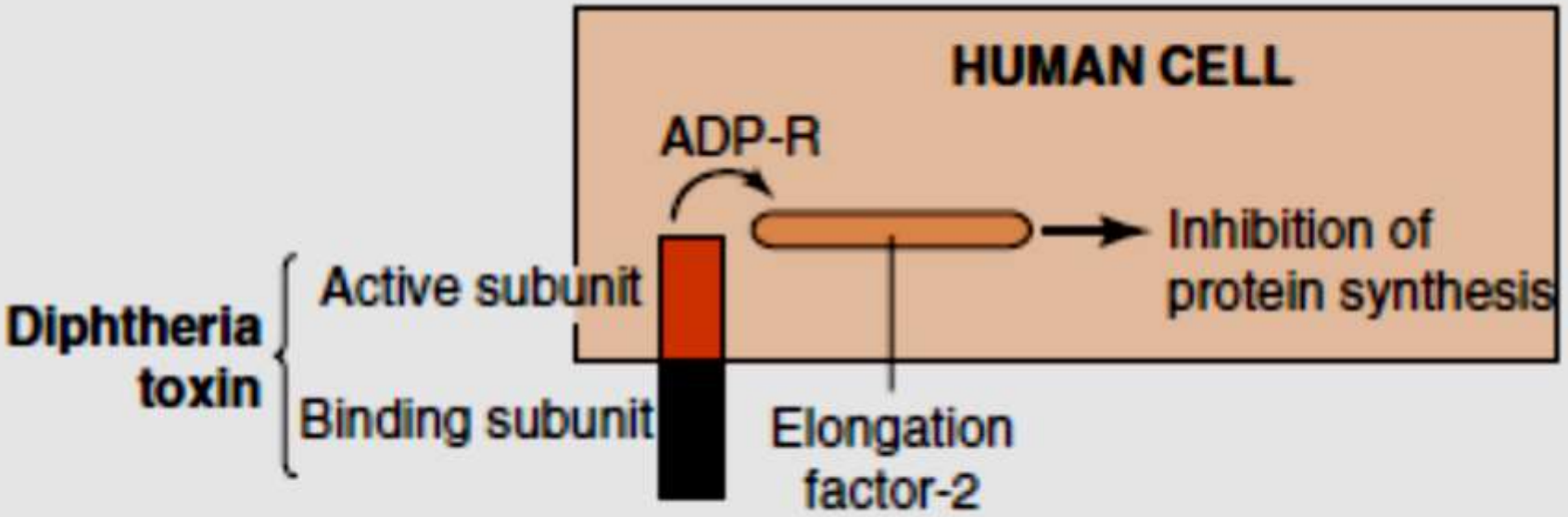
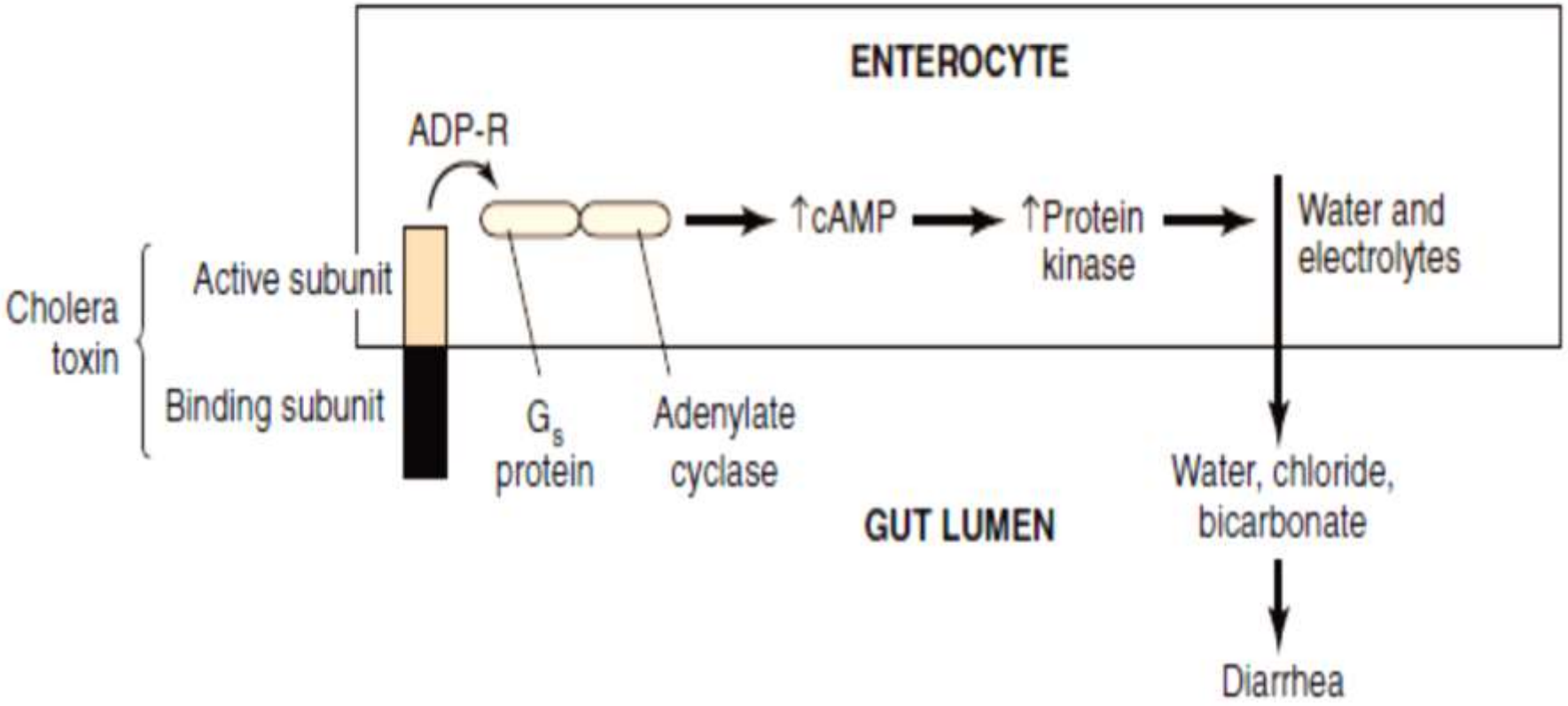


FIGURE 7–1 Mode of action of diphtheria toxin. The toxin binds to the cell surface via its binding subunit, and the active subunit enters the cell. The active subunit is an enzyme that catalyzes the addition of ADP-ribose (ADP-R) to elongation factor-2 (EF-2). This inactivates EF-2, and protein synthesis is inhibited.

Gram Negative Exotoxins

- Heat-labile enterotoxin of *E. coli* (AMP)
- Heat-stable enterotoxin of *E. coli* (GMP)
- Verotoxins of *E. coli* O157:H7 (HUS)
- Shiga toxin (verotoxin)
- Pertussis toxin (ADP-ribosylation)



Mode of action of *E. coli* and *Vibrio cholerae* enterotoxins

Immunopathogenesis

- ***Rheumatic fever (RF):***

  - certain M protein on pili of *S. pyogenes*

- ***Acute glomerulonephritis (AGN):***

  - certain M protein on pili of *S. pyogenes*

Bacterial Infections Associated with Cancer

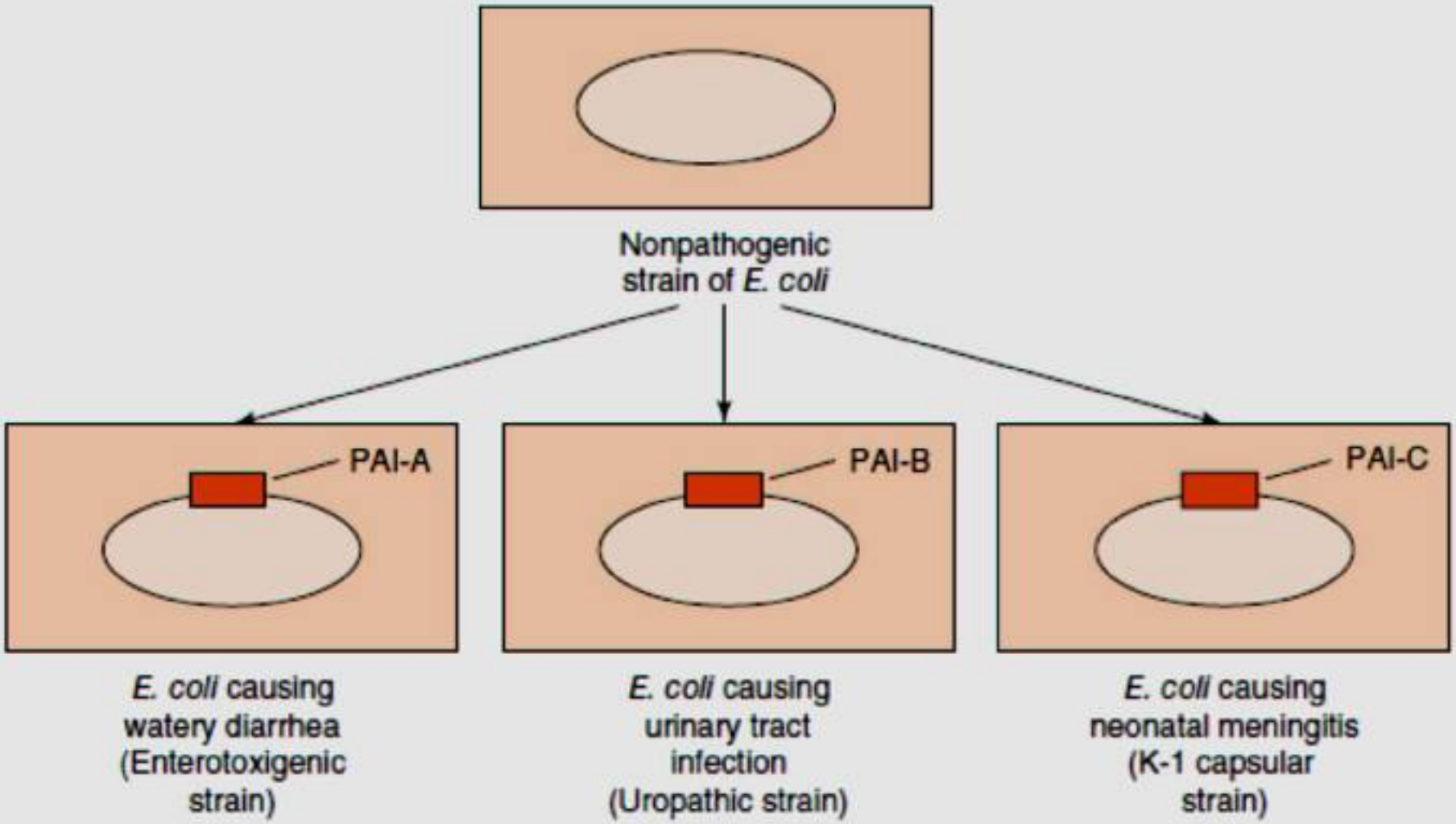
- ***H. pylori :***

  Gastric carcinoma and gastric mucosal-associated lymphoid tissue (MALT) lymphoma

- ***C. Jejuni:***

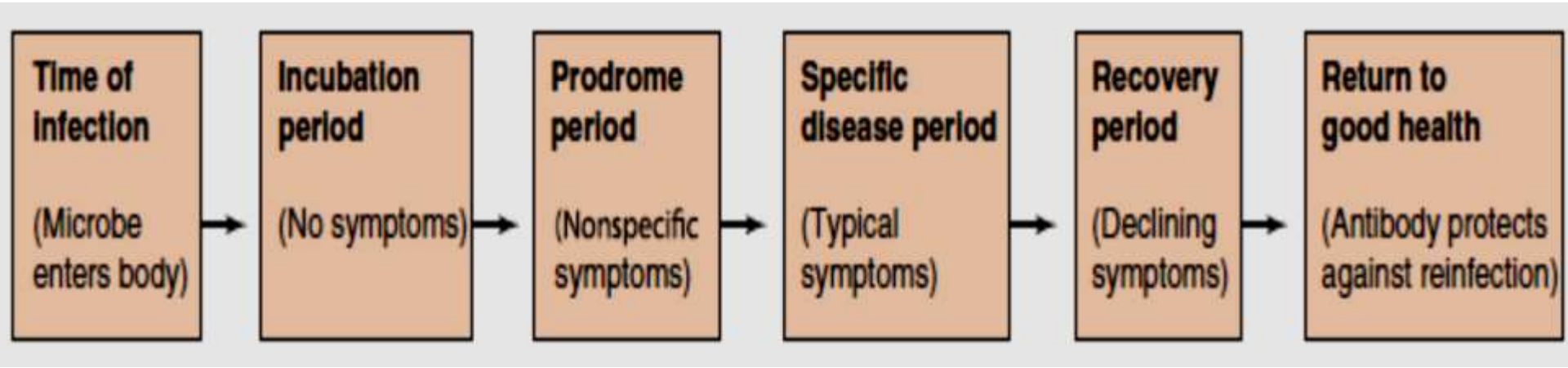
  MALT lymphoma of the small intestine

DIFFERENT STRAINS OF THE SAME BACTERIA CAN PRODUCE DIFFERENT DISEASES



Typical Stages of an Infectious Disease

- **Incubation period**
- **Prodrome period**
- **Specific-illness period**
- **Recovery period (convalescence)**
  - ✓Chronic carriers
  - ✓Latent infection
  - ✓Subclinical



Typical stages of an infectious disease

Portals of Exit

- **Respiratory tract**
  - Coughing, sneezing
- **Gastrointestinal tract**
  - Feces, saliva
- **Genitourinary tract**
  - Urine, vaginal secretions
- **Skin**
- **Blood**
  - Biting arthropods, needles/syringes

Did the organism isolated from the patient actually cause the disease?

➢ Koch's postulates:

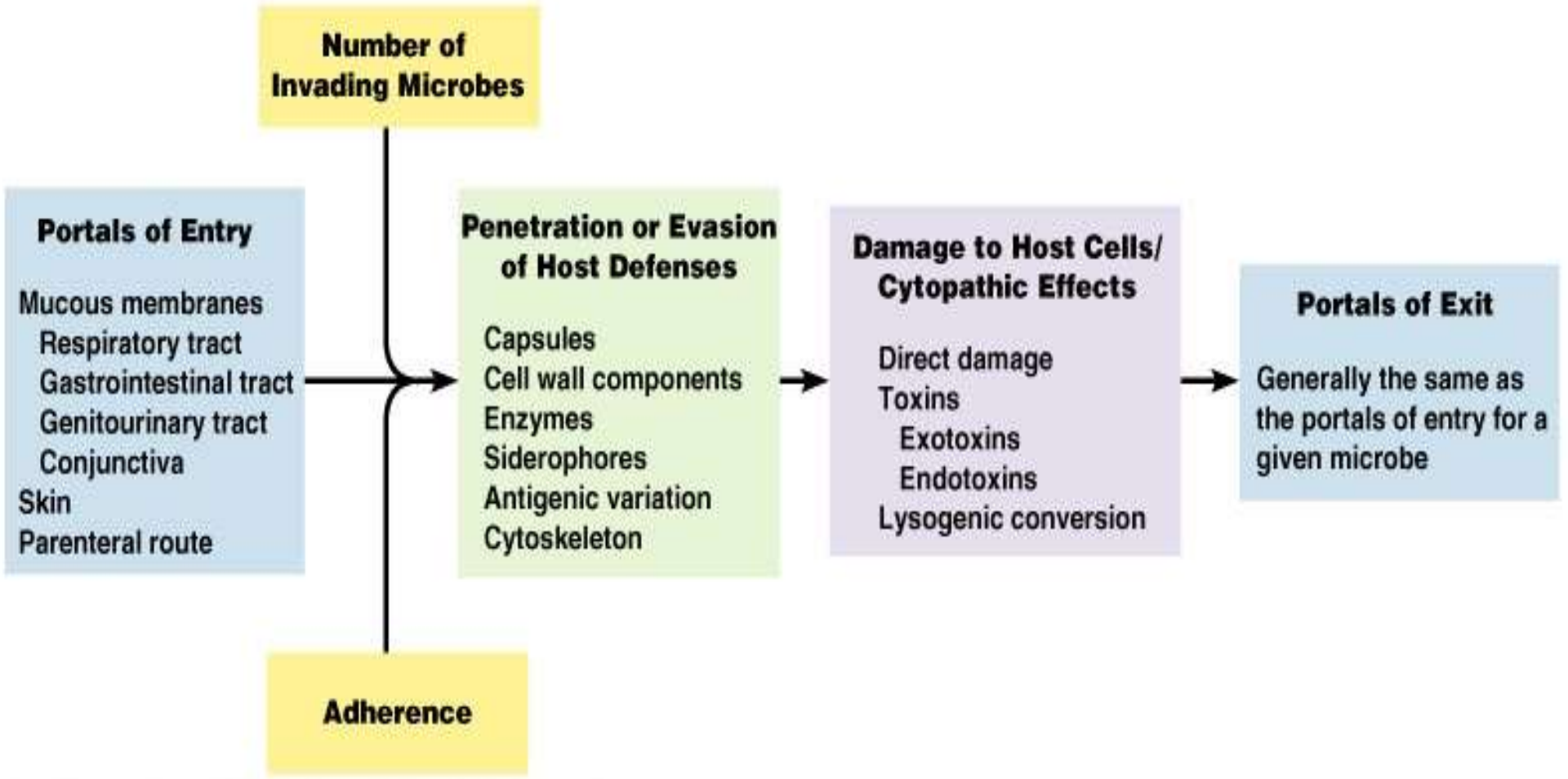
- (1) The organism must be isolated from every patient with the disease.
- (2) The organism must be isolated free from all other organisms and grown in pure culture *in vitro*.
- (3) The pure organism must cause the disease in a healthy, susceptible animal.
- (4) The organism must be recovered from the inoculated animal.

✓asymptomatic carriers (as salmonella)

✓uncultivable (as *Treponema pallidum*)

✓exposure but no illness (as *M. tuberculosis*)

Mechanisms of Pathogenicity



TORTORA • FUNKE • CASE

Microbiology

AN INTRODUCTION

EIGHTH EDITION

Host Defenses

PowerPoint® Lecture Slide Presentation prepared by Christine L. Case

Nonspecific Defenses of the Host

- **Susceptibility** — Lack of resistance to a disease
- **Resistance** — Ability to ward off disease
- **Nonspecific resistance** — Defenses against any pathogen
- **Specific resistance** — Immunity, resistance to a specific pathogen

Host Defenses

Nonspecific Resistance		Specific Resistance (Responses of the Immune System, Chapter 17)
First line of defense	**Second line of defense**	**Third line of defense**
• Intact skin • Mucous membranes and their secretions • Normal microbiota	• Phagocytic white blood cells • Inflammation • Fever • Antimicrobial substances	• Specialized lymphocytes: B cells and T cells • Antibodies

Mechanical Factors

- **Skin**
- Epidermis consists of tightly packed cells with Keratin, a protective protein
- **Mucous membranes**
- **Ciliary escalator:** Microbes trapped in mucus are transported away from the lungs
- Lacrimal apparatus: Washes eye
- Saliva: Washes microbes off
- Urine: Flows out
- Vaginal secretions: Flow out

Chemical Factors

- Fungistatic fatty acid in sebum (ring worm)
- Low pH (3-5) of skin
- Lysozyme in perspiration, tears, saliva, and tissue fluids
- Low pH (1.2-3.0) of gastric juice
- Transferrin in blood find iron
- Defensins (*highly positively charged peptide*)

Normal Microbiota

- Microbial antagonism/competitive exclusion: Normal microbiota compete with pathogens.

White Blood Cells

- Neutrophils: Phagocytic
- Basophils: Produce histamine
- Eosinophils: Toxic to parasites, some phagocytosis
- Monocytes: Phagocytic as mature macrophages
- Fixed macrophages in lungs, liver, bronchi
- Wandering macrophages roam tissues
- Lymphocytes: Involved in specific immunity

Differential White Cell Count

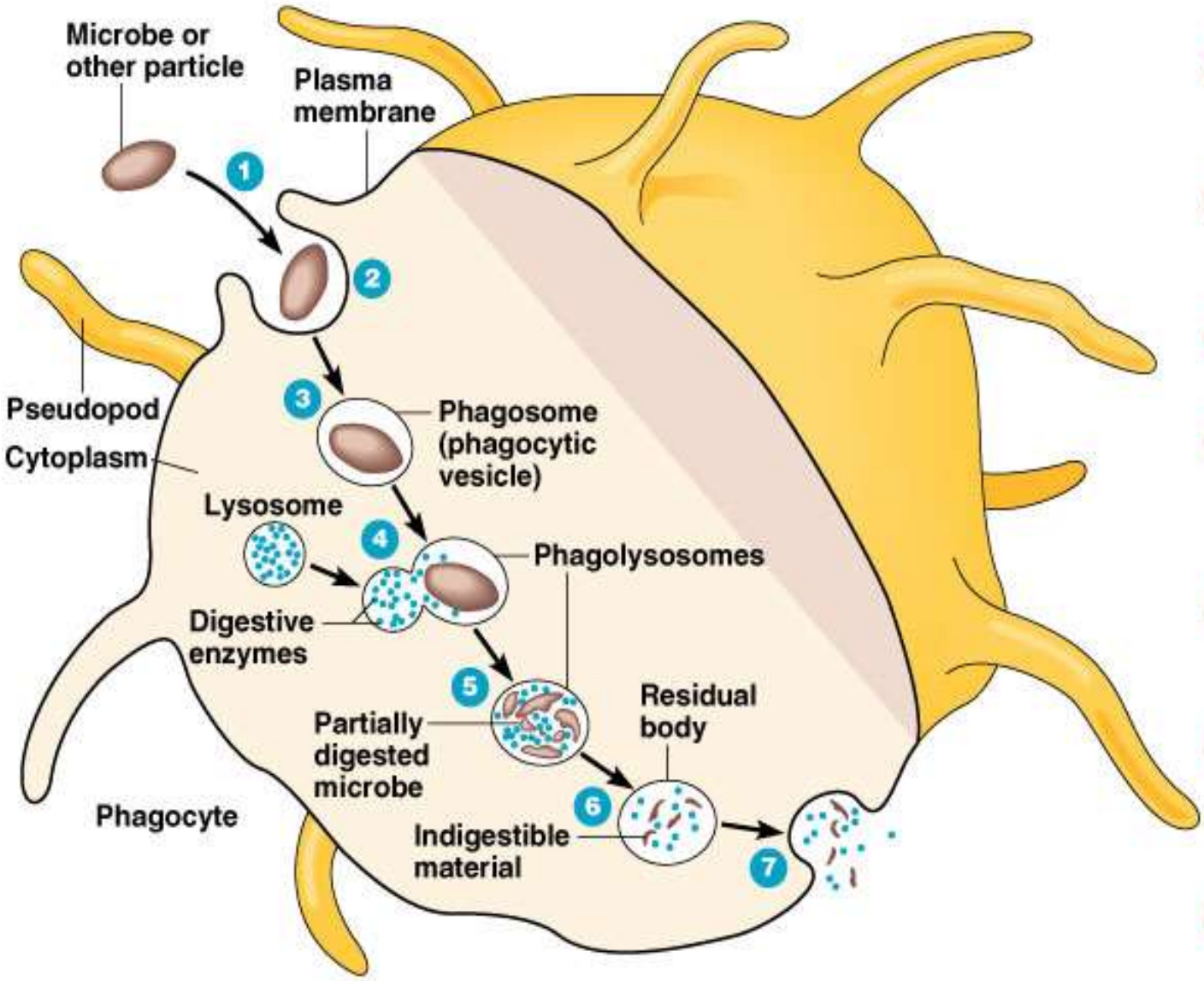
- Percentage of each type of white cell in a sample of 100 white blood cells

Neutrophils	**60-70%**
Basophils	0.5-1%
Eosinophils	2-4%
Monocytes	3-8%
Lymphocytes	**20-25%**

Phagocytosis

- *Phago*: eat
- *Cyte*: cell
- Ingestion of microbes or particles by a cell, performed by phagocytes

Phagocytosis



(a) Phases of phagocytosis

1. Chemotaxis and adherence of microbe to phagocyte.
2. Ingestion of microbe by phagocyte.
3. Formation of a phagosome.
4. Fusion of the phagosome with a lysosome to form a phagolysosome.
5. Digestion of ingested microbe by enzymes.
6. Formation of residual body containing indigestible material.
7. Discharge of waste materials.

Microbial Evasion of Phagocytosis

• Inhibit adherence: M protein, capsules	*Streptococcus pyogenes, S. pneumoniae*
• Kill phagocytes: Leukocidins	*Staphylococcus aureus*
• Lyse phagocytes: Membrane attack complex	*Listeria monocytogenes*
• Escape phagosome	*Shigella*
• Prevent phagosome-lysosome fusion	HIV
• Survive in phagolysosome	*Coxiella burnetti*

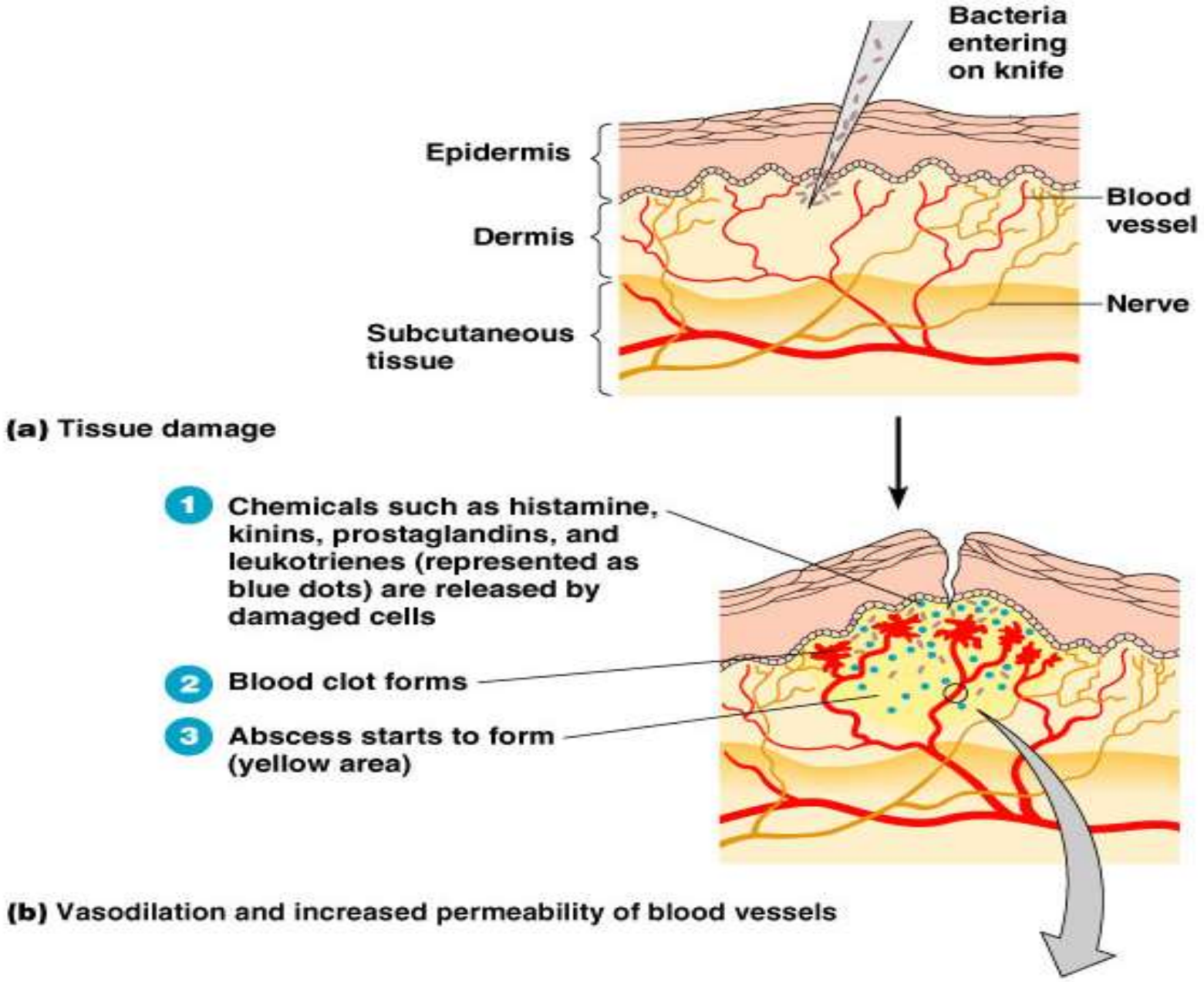
Inflammation

- Redness
- Pain
- Heat
- Swelling (edema)
- Acute-phase proteins activated (complement, cytokine, kinins)
- Vasodilation (histamine, kinins, prostaglandins, leukotrienes)
- Margination and emigration of WBCs
- Tissue repair

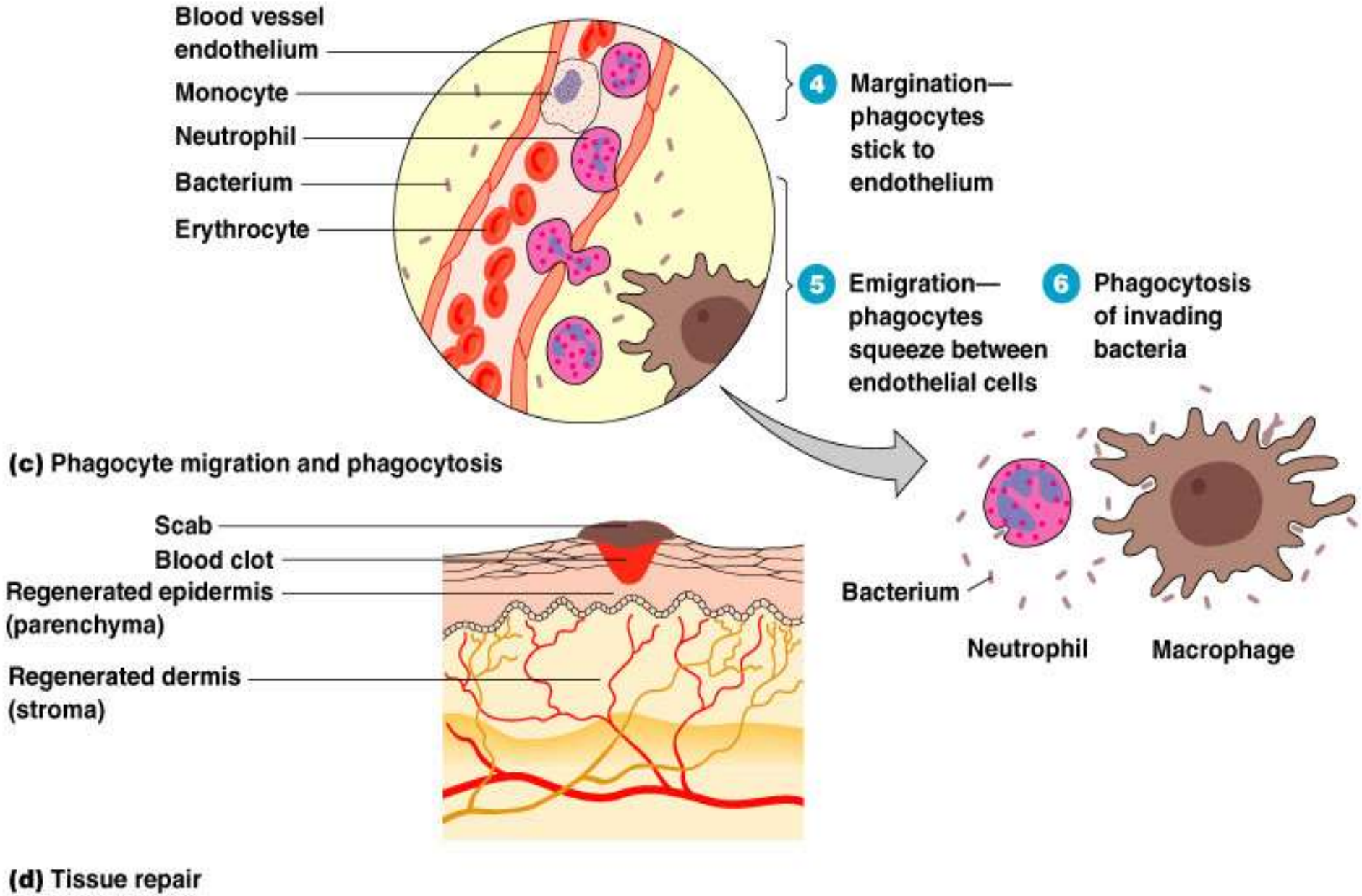
Chemicals Released by Damaged Cells

➢ **Histamine**	**Vasodilation, increased permeability of blood vessels**
➢ **Kinins**	**Vasodilation, increased permeability of blood vessels**
➢ **Prostaglandins**	**Intensity histamine and kinin effect**
➢ **Leukotrienes**	**Increased permeability of blood vessels, phagocytic attachment**

Inflammation

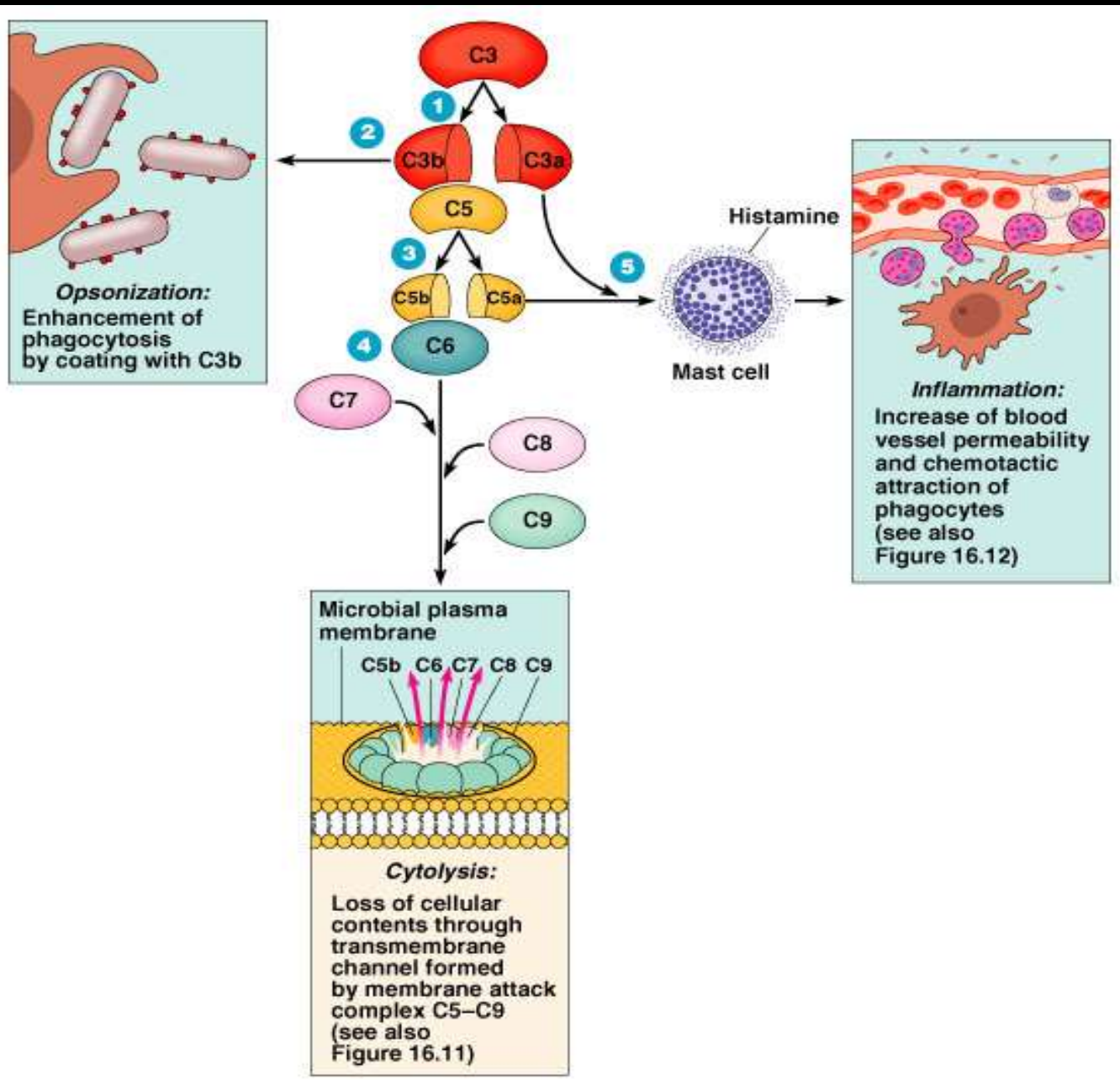


Inflammation



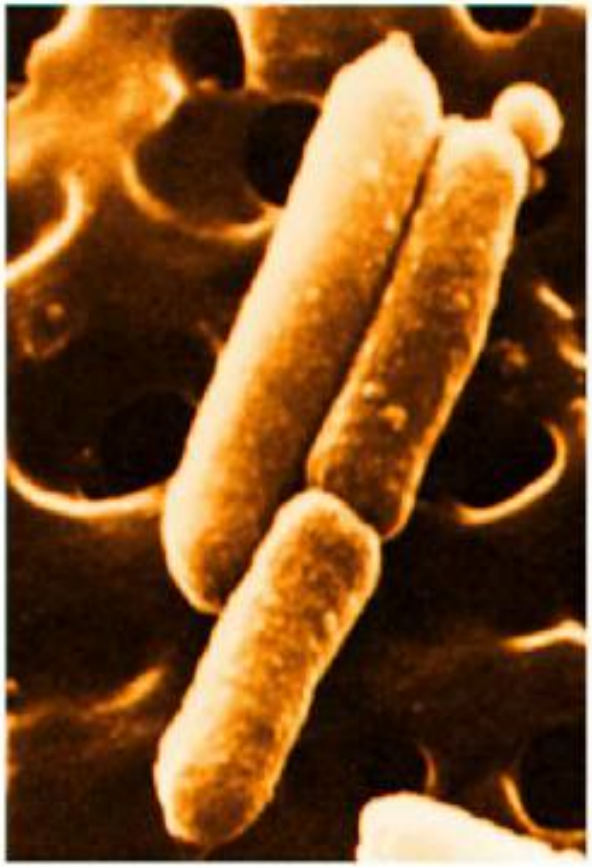
The Complement System

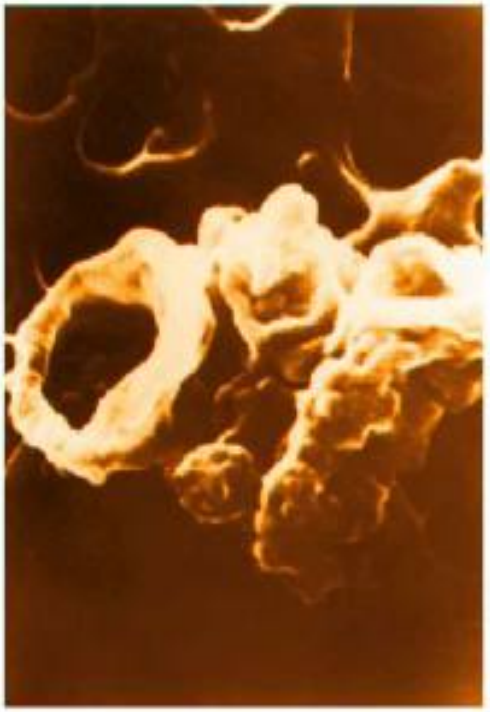
- Serum proteins activated in a cascade.



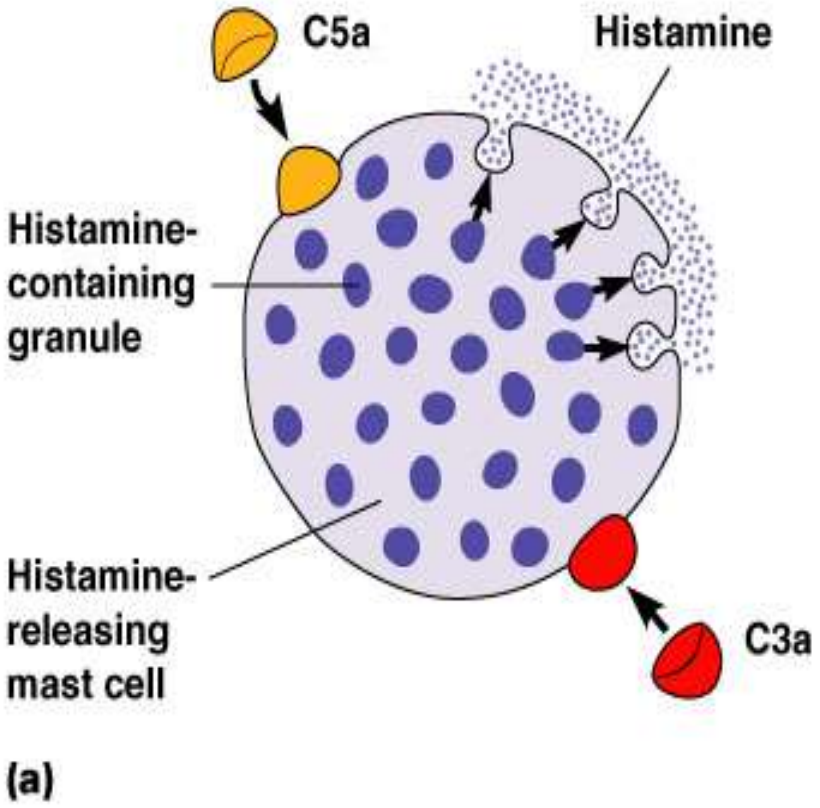
Effects of Complement Activation

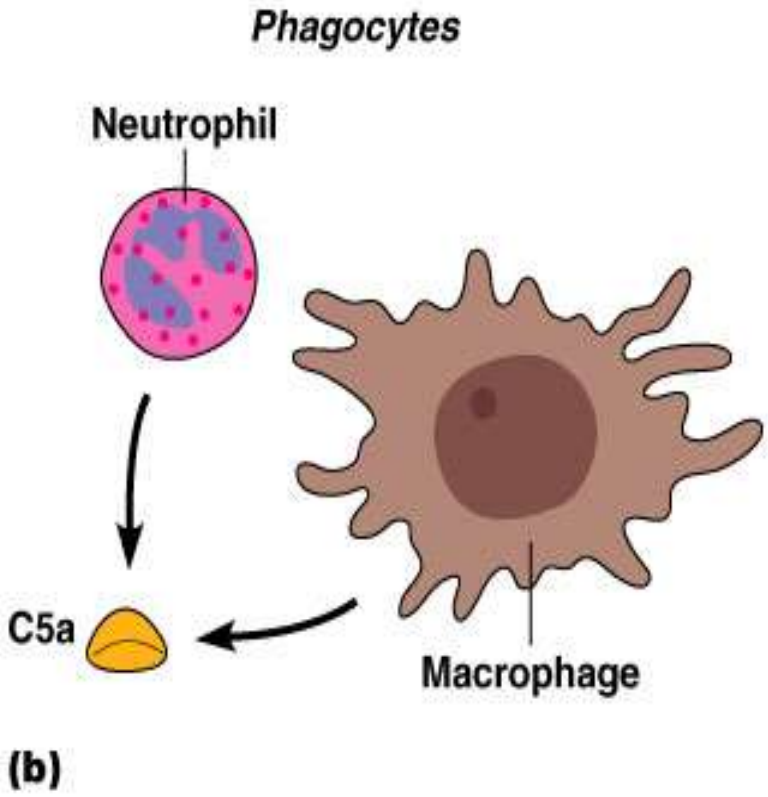
- Opsonization or immune adherence: enhanced phagocytosis
- Membrane attack complex: cytolysis
- Attract phagocytes



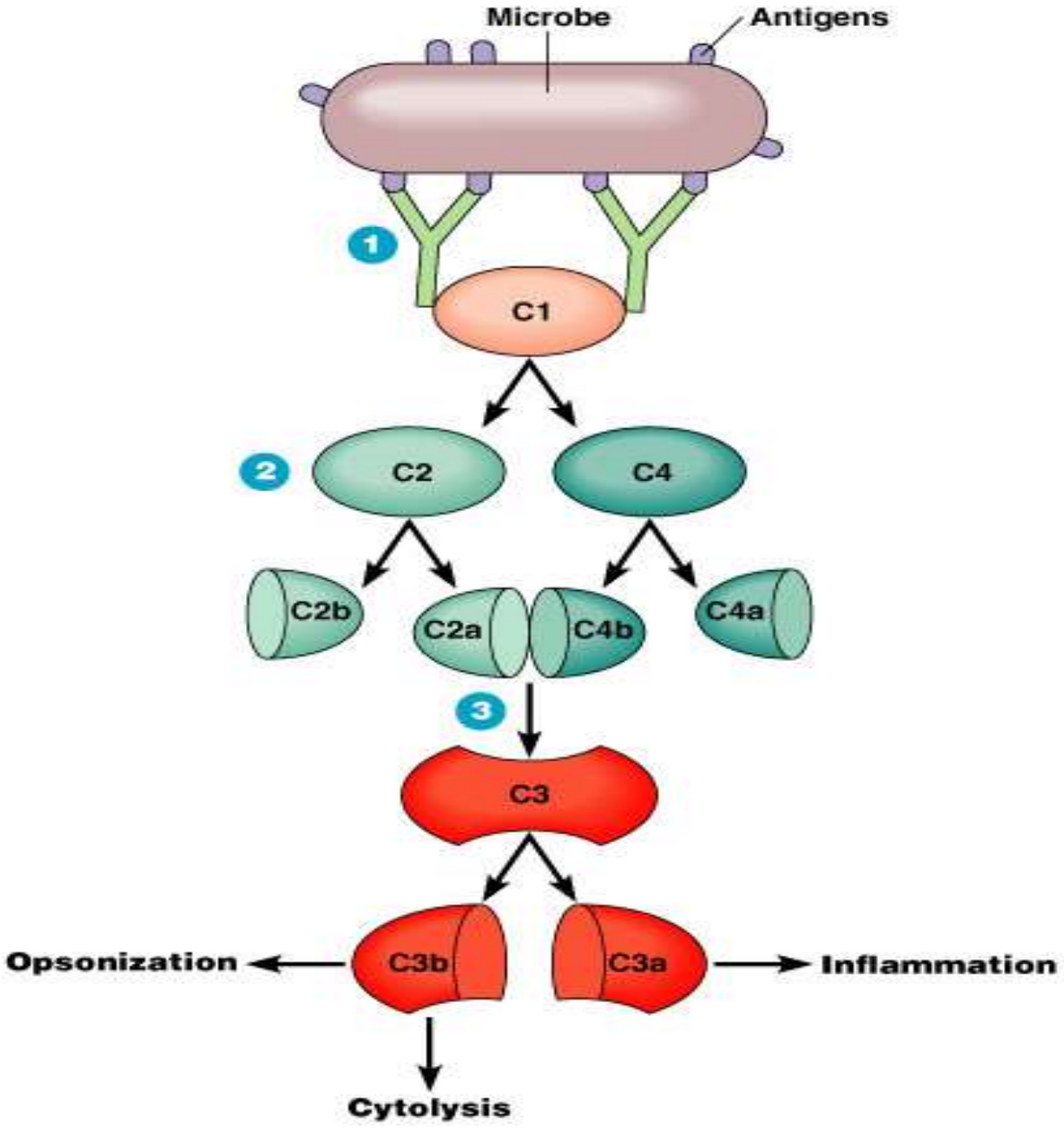


Effects of Complement Activation

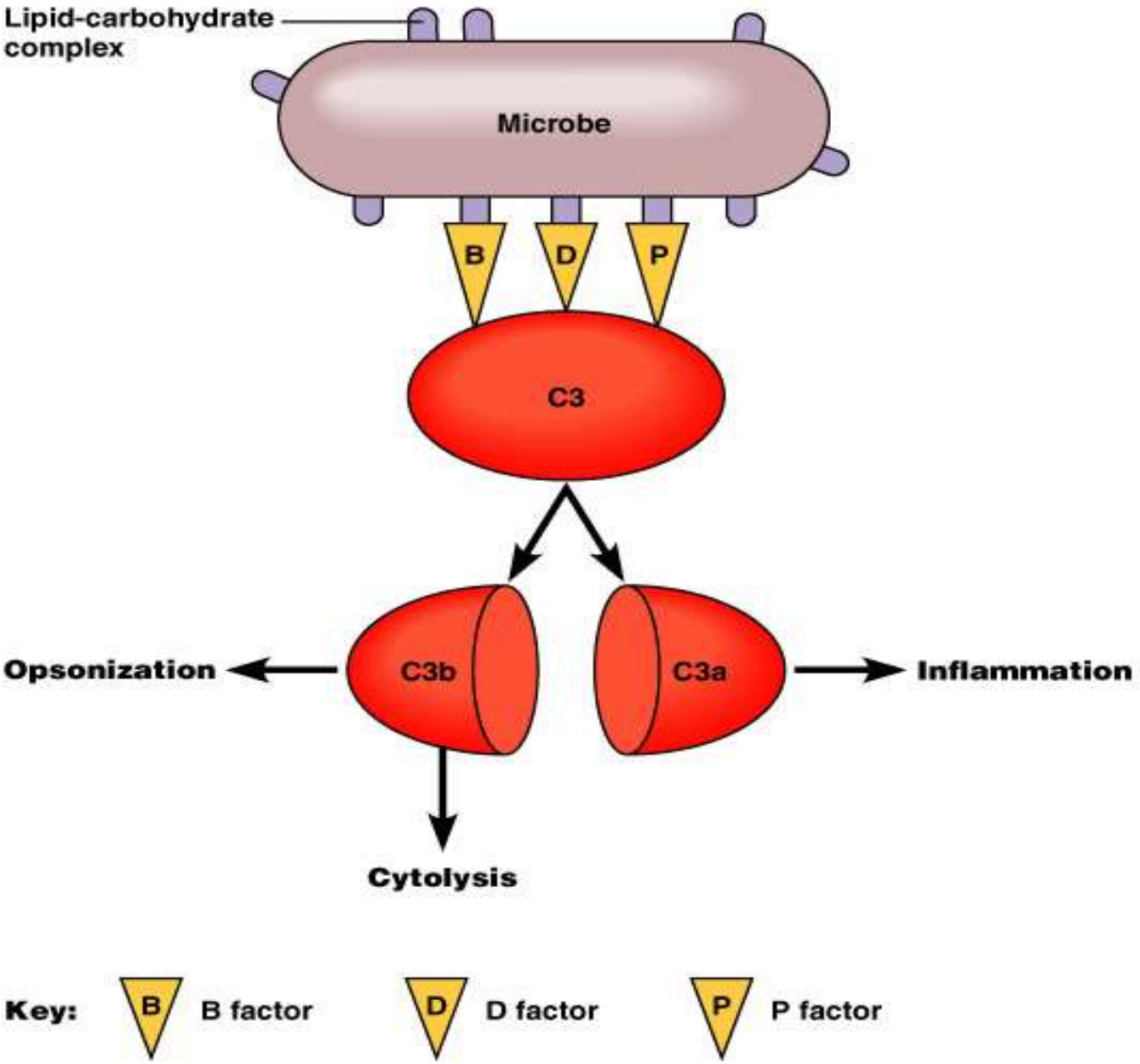




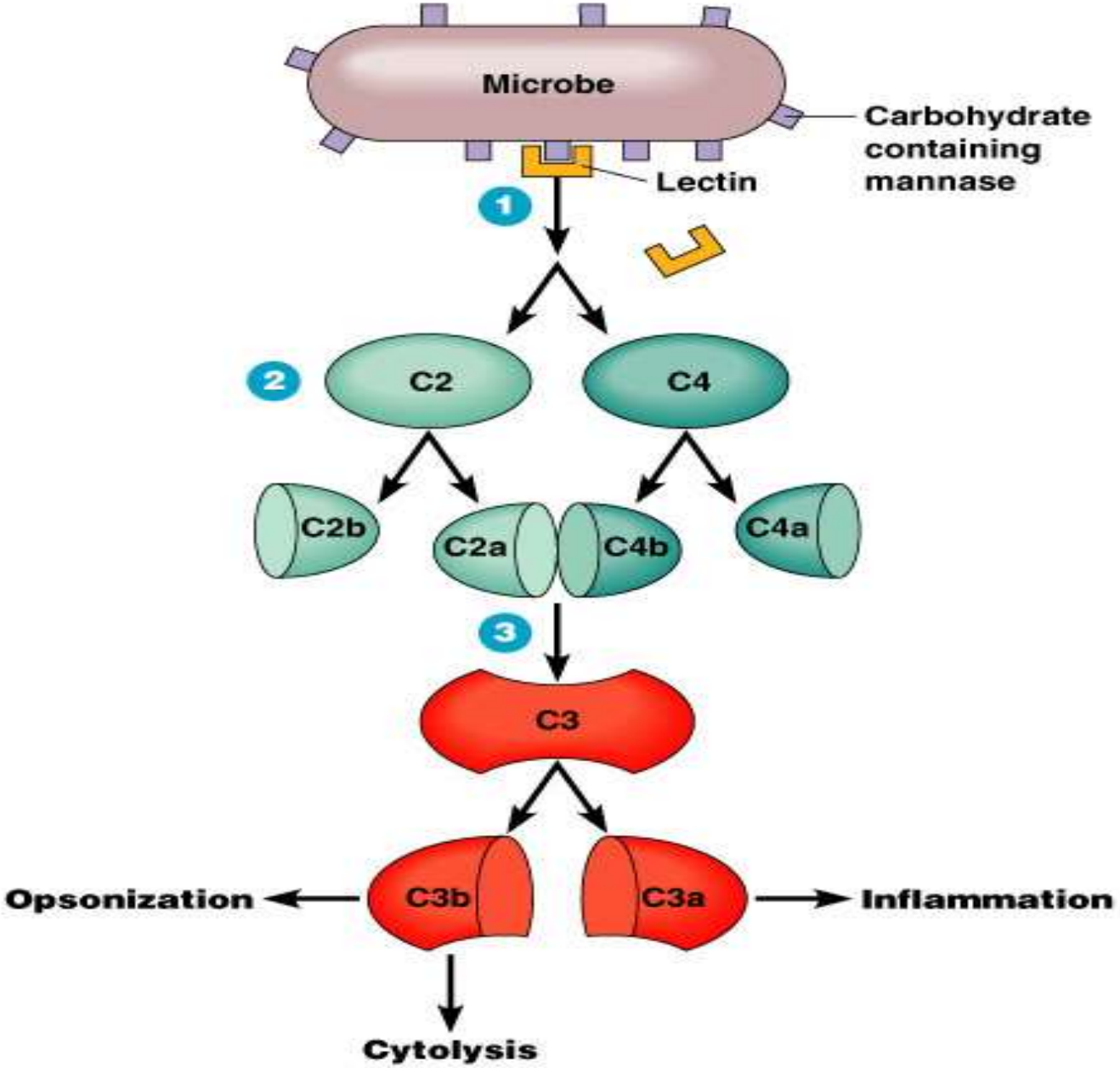
Classical Pathway



Alternative Pathway



Lectin Pathway



Some bacteria evade complement

- Capsules prevent C activation
- Surface lipid-carbohydrates prevent MAC formation
- Enzymatic digestion of C5a

Interferons (IFNs)

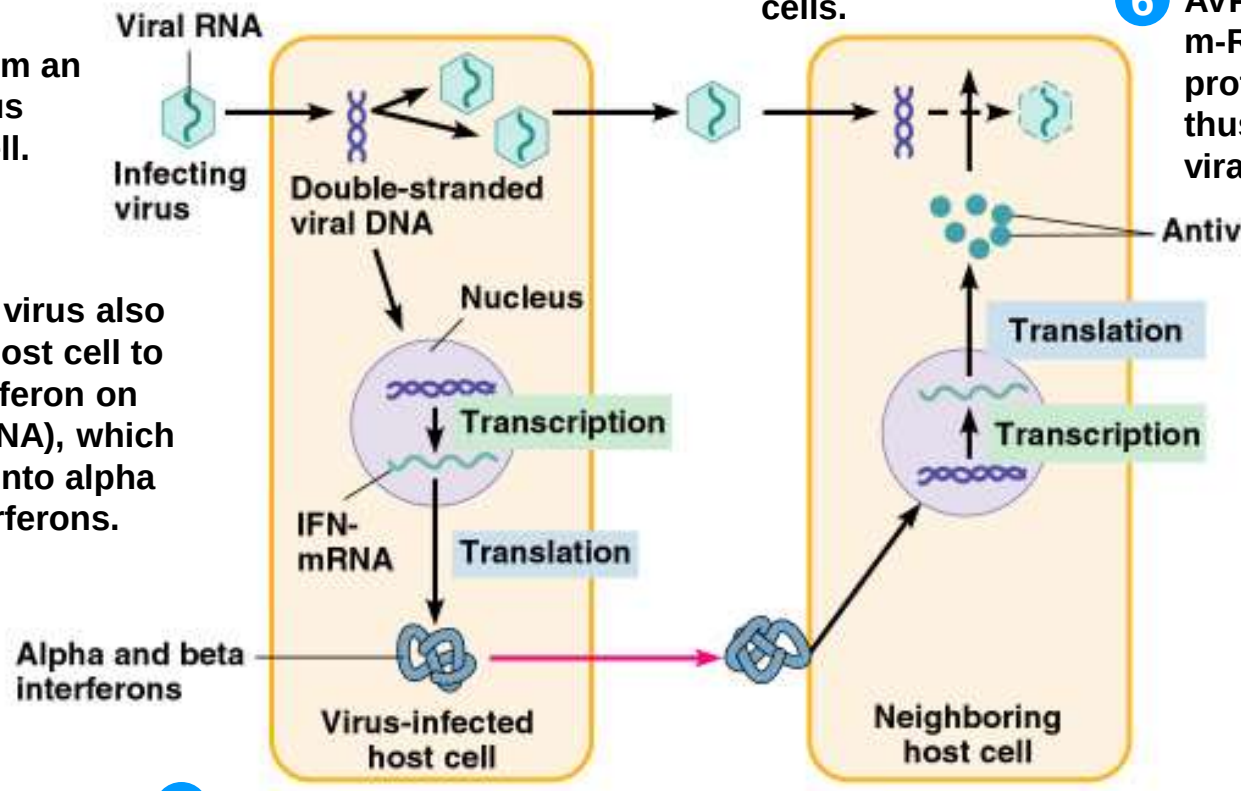
- **Alpha IFN & Beta IFN:** Cause cells to produce antiviral proteins that inhibit viral replication
- **Gamma IFN:** Causes neutrophils and macrophages to phagocytize bacteria

Interferons (IFNs)

1. Viral RNA from an infecting virus enters the cell.

2. The infecting virus replicates into new viruses.

3. The infecting virus also induces the host cell to produce interferon on RNA (IFN-mRNA), which is translated into alpha and beta interferons.



4. Interferons released by the virus-infected host cell bind to plasma membrane or nuclear membrane receptors on uninfected neighboring host cells, inducing them to synthesize antiviral proteins (AVPs). These include oligoadenylate synthetase, and protein kinase.

5. New viruses released by the virus-infected host cell infect neighboring host cells.

6. AVPs degrade viral m-RNA and inhibit protein synthesis and thus interfere with viral replication.

Reduced Phagocytosis Predisposes to Bacterial Infections

- Repeated infections as in CGD (defect in NADPH), Chediak-Higashi syndrome (no lysozyme fusion).
- PMNKs less than 500/µl
- Splenectomy
- D.M.

Fever: Abnormally High Body Temperature

- Hypothalamus normally set at 37°C
- Gram-negative endotoxin cause phagocytes to release interleukin 1
- Hypothalamus releases prostaglandins that reset the hypothalamus to a high temperature
- Body increases rate of metabolism and shivering to raise temperature
- When IL-1 is eliminated, body temperature falls.

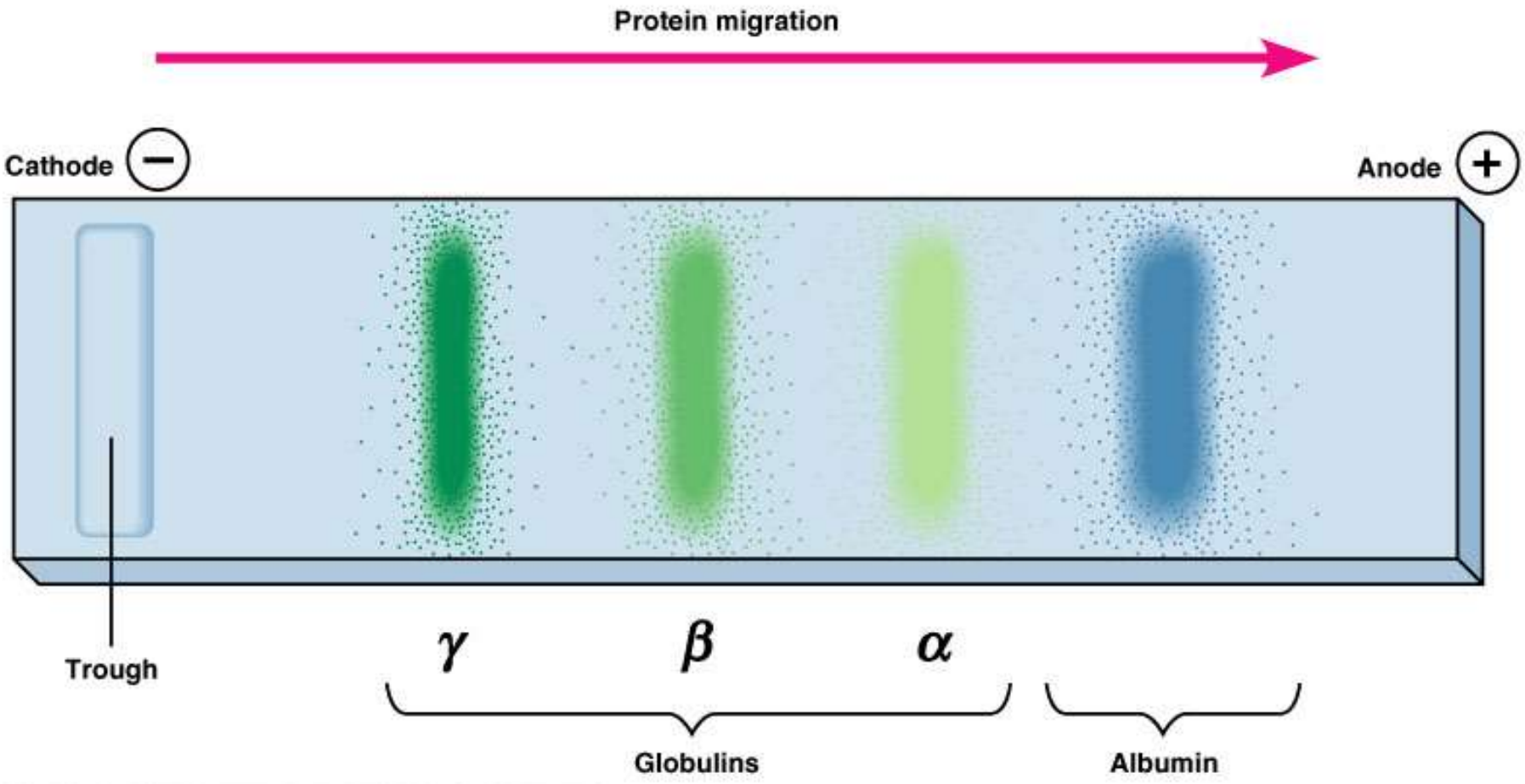
Specific Defenses of the Host: The Immune Response

- Innate (nonspecific) — Defenses against any pathogen
- Immunity — Specific antibody and lymphocyte response to an antigen
- Antigen (Ag) — A substances that causes the body to produce specific antibodies or sensitized T cells
- Antibody (Ab) — Proteins made in response to an antigen

Terminology

- Serology — Study of reactions between antibodies and antigens
- Antiserum — Generic term for serum because it contains Ab
- Globulins — Serum proteins
- Gamma (γ) globulin — Serum fraction containing Ab

Serum Proteins



The Immune Response

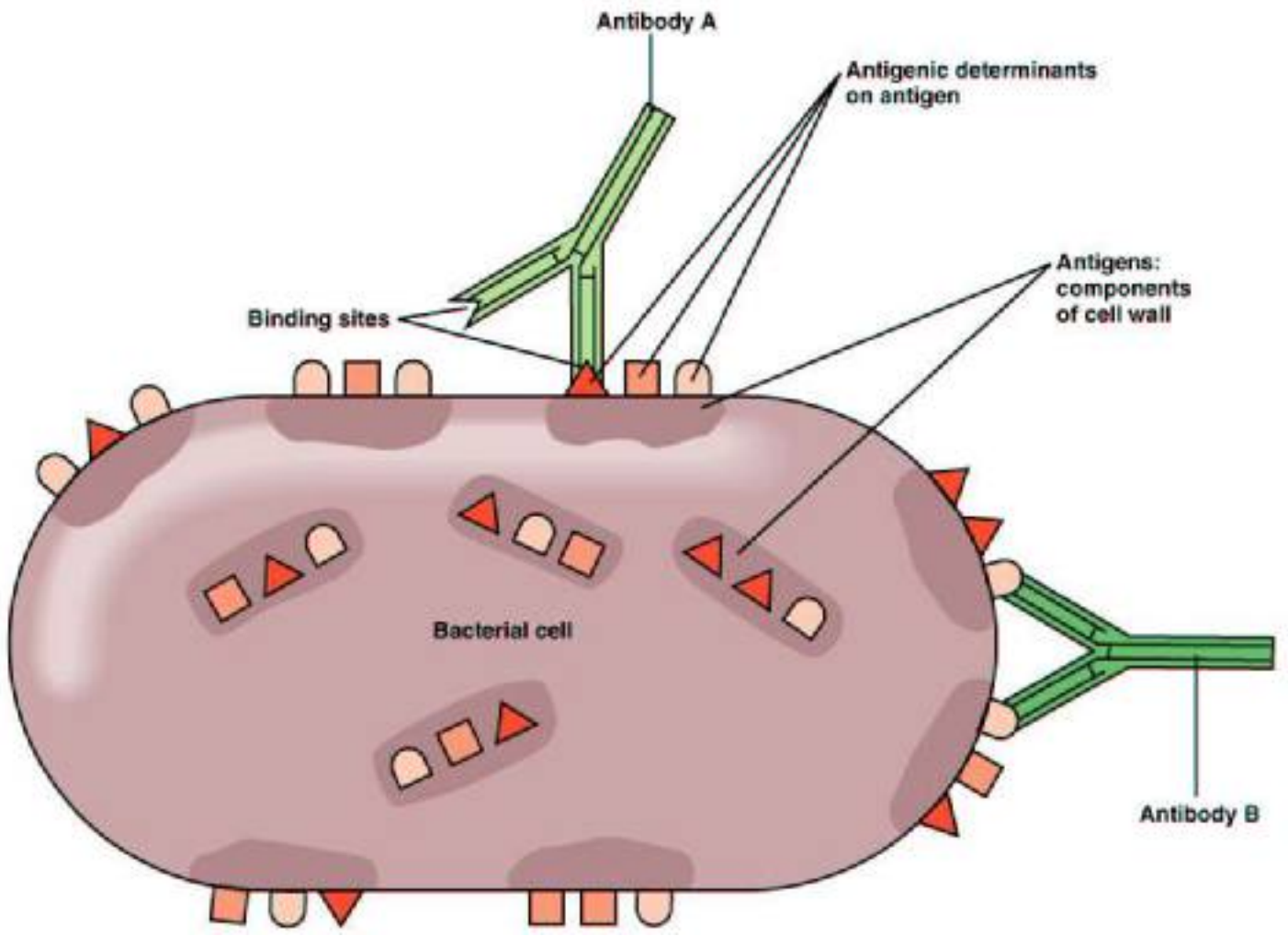
- Acquired immunity — Developed during an individual's lifetime
- Humoral immunity — Involves Ab produced by B cells
- Cell-mediated immunity — Involves T cells

Acquired (adaptive) Immunity

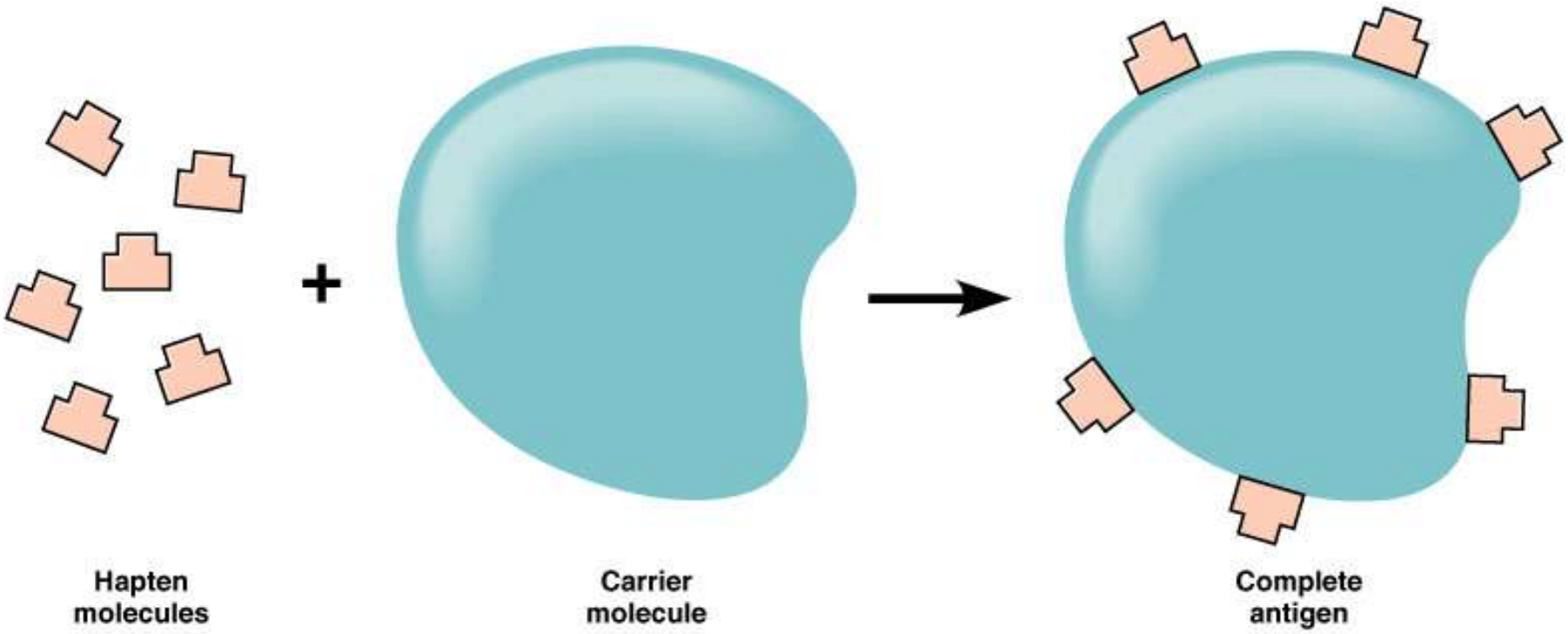
- Naturally acquired active immunity
  - Resulting from infection
- Naturally acquired passive immunity
  - Transplacental or via colostrum
- Artificially acquired active immunity
  - Injection of Ag (vaccination)
- Artificially acquired passive immunity
  - Injection of Ab

Antigenic Determinants

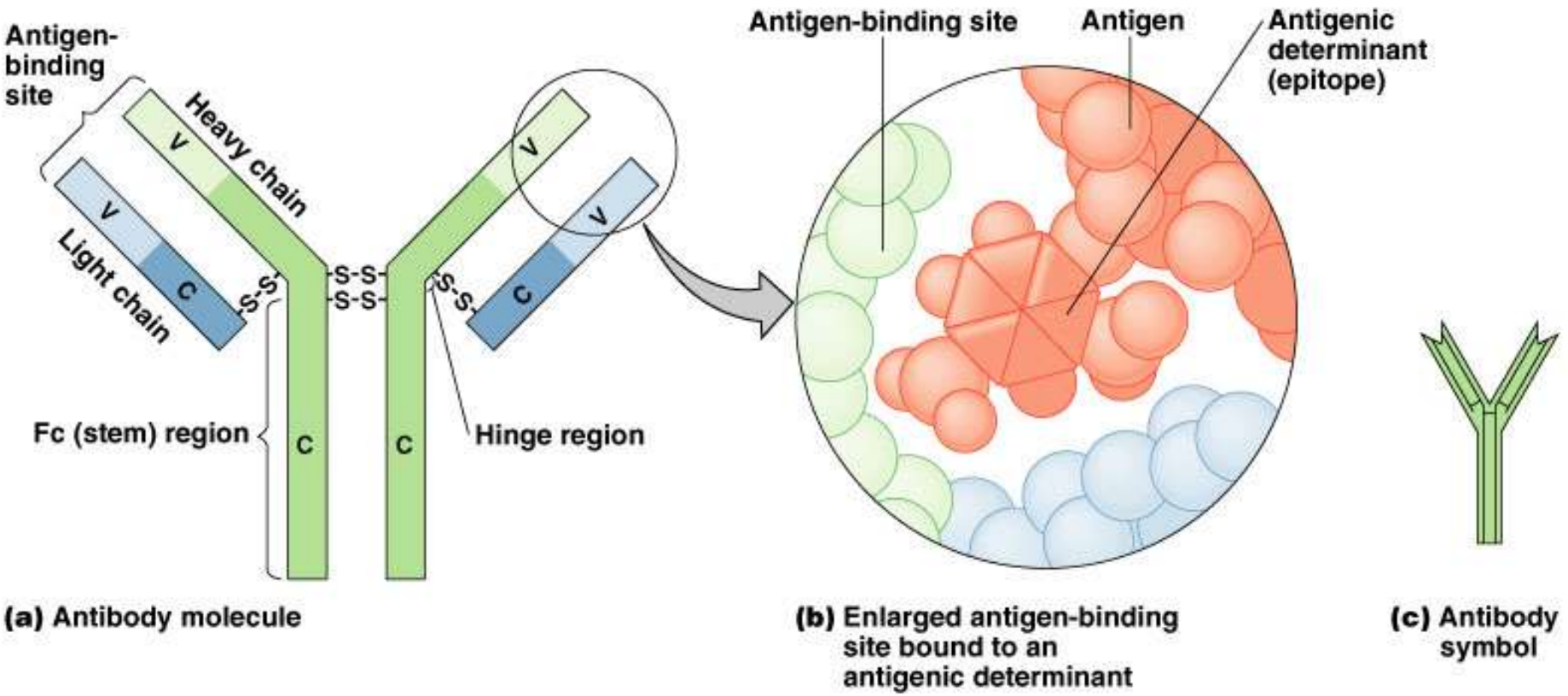
- Antibodies recognize and react with antigenic determinants or epitopes.



Haptens



Antibody Structure

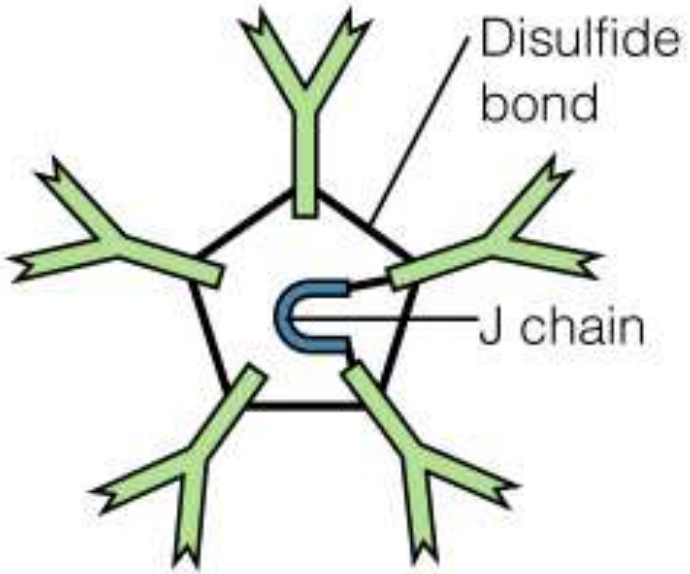


IgG antibodies

- Monomer
- 80% of serum antibodies
- Fix complement
- In blood, lymph, intestine
- Cross placenta
- Enhance phagocytosis; neutralize toxins & viruses; protects fetus & newborn
- Half-life = 23 days

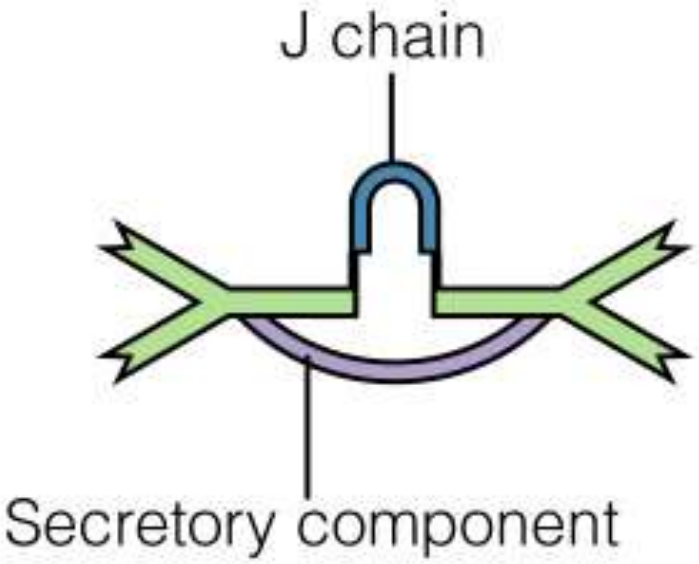
IgM antibodies

- Pentamer
- 5-10% of serum antibodies
- Fix complement
- In blood, lymph, on B cells
- Agglutinates microbes; first Ab produced in response to infection
- Half-life = 5 days



IgA antibodies

- Dimer
- 10-15% of serum antibodies
- In secretions
- Mucosal protection
- Half-life = 6 days

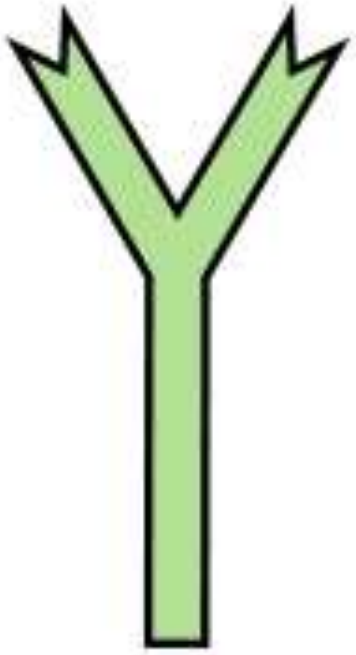


IgD antibodies

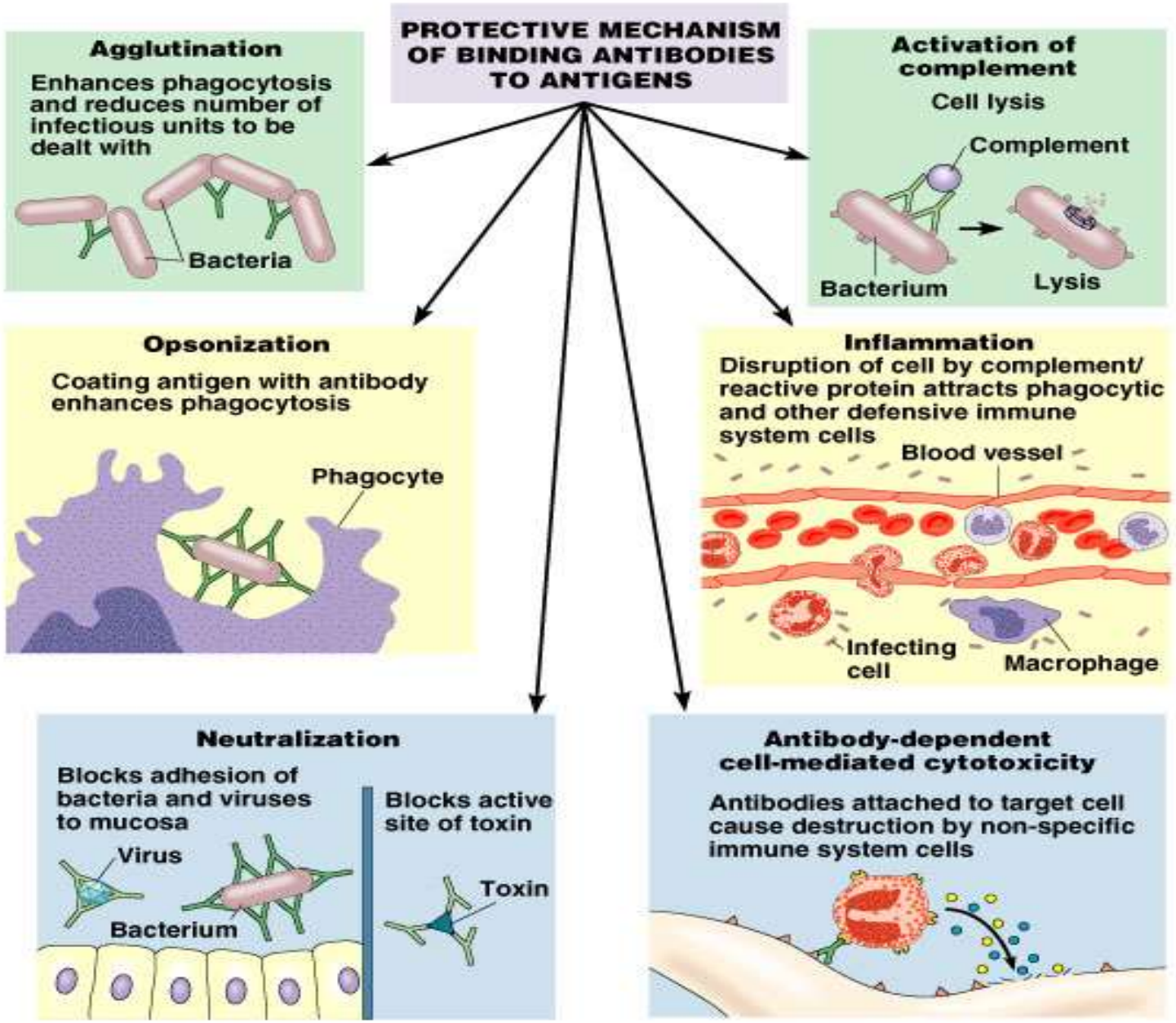
- Monomer
- 0.2% of serum antibodies
- In blood, lymph, on B cells
- On B cells, initiate immune response
- Half-life = 3 days

IgE antibodies

- Monomer
- 0.002% of serum antibodies
- On mast cells and basophils, in blood
- Allergic reactions; lysis of parasitic worms
- Half-life = 2 days

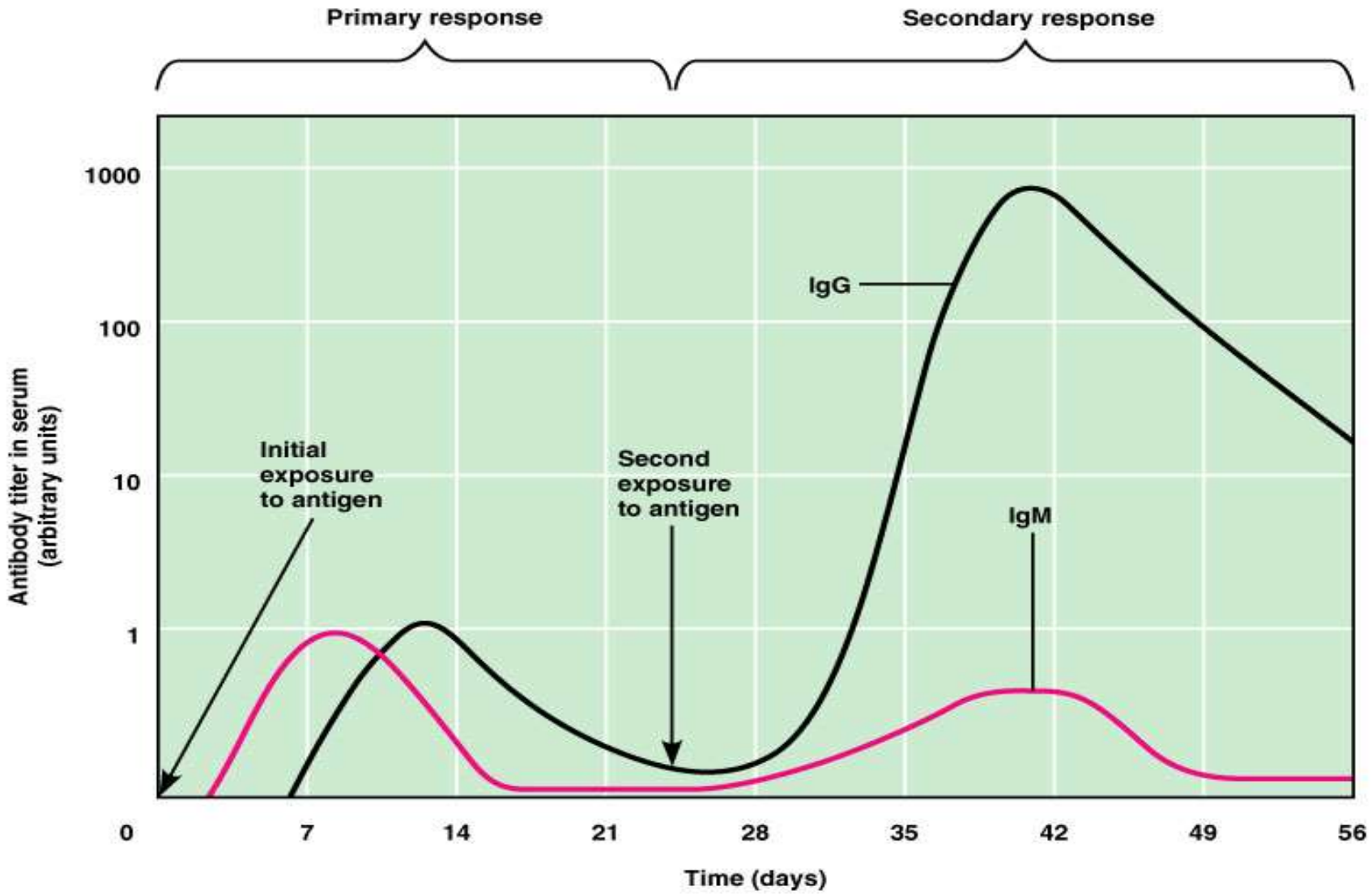


The Results of Ag-Ab Binding



Antibody titer:

- Is the amount of Ab in serum



Immune system cells communicate via cytokines

- Interleukin-1 — Stimulates T_H cells
- Interleukin-2 — Activates T_H, B, T_C, and NK cells
- Interleukin-12 — Differentiation of CD4 cells
- γ-Interferon — Increase activity of macrophages
- Chemokines — Cause leukocytes to move to an infection

Cell-Mediated Immunity

- Specialized lymphocytes, mostly T cells, respond to intracellular Ags
- After differentiating in the thymus, T cells migrate to lymphoid tissue
- T cells differentiate into effector T cells when stimulated by an Ag
- Some effector T cells become memory cells

Pathogens entering the gastrointestinal or respiratory tracts pass through:

- **M (microfold) cells** in
- **Peyer's patches** which contains
- **Dendritic cells** which are **antigen-presenting cells (APS)** and
- **T cells**

Dendritic cells present antigens

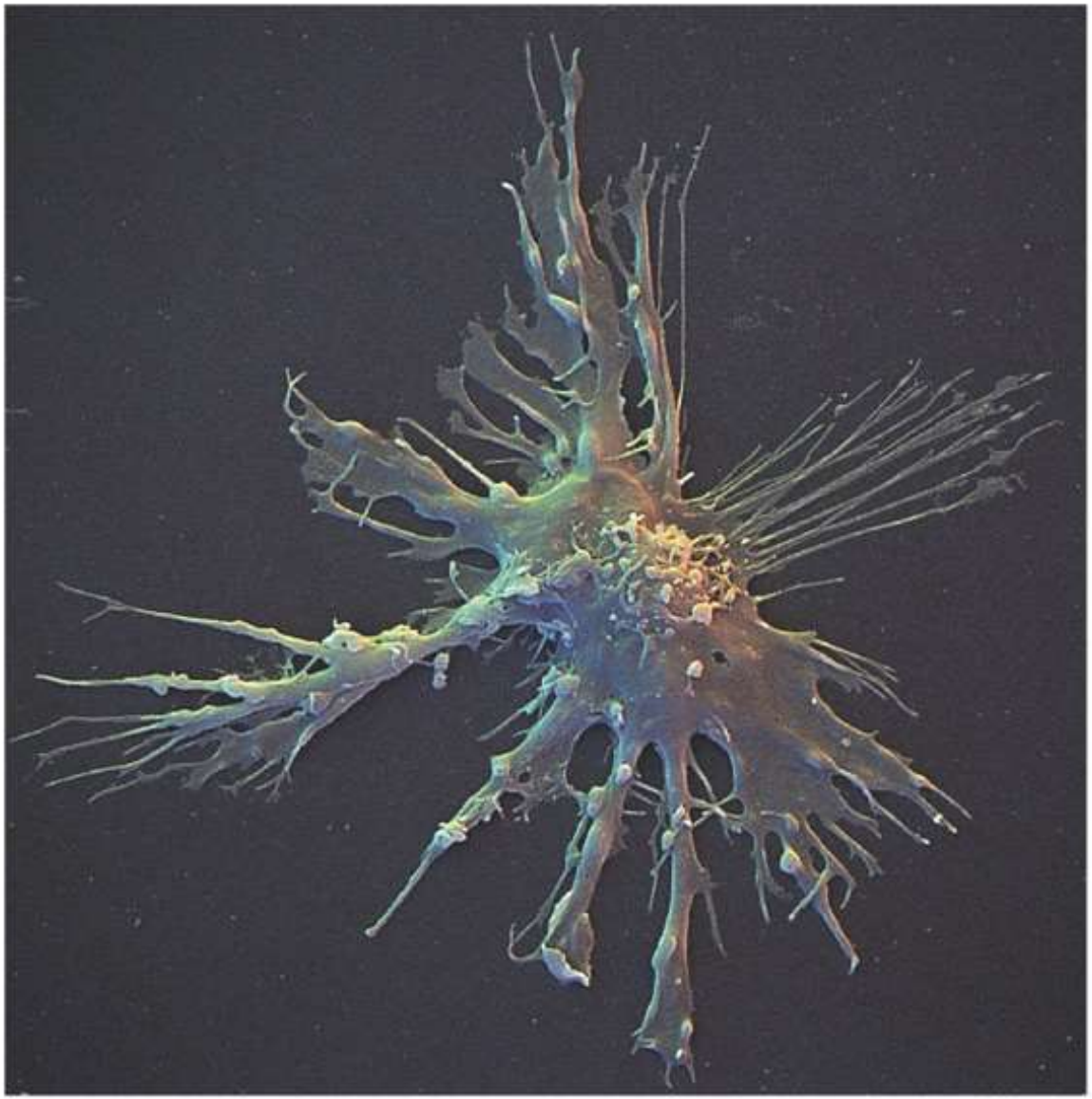


Figure 17.12

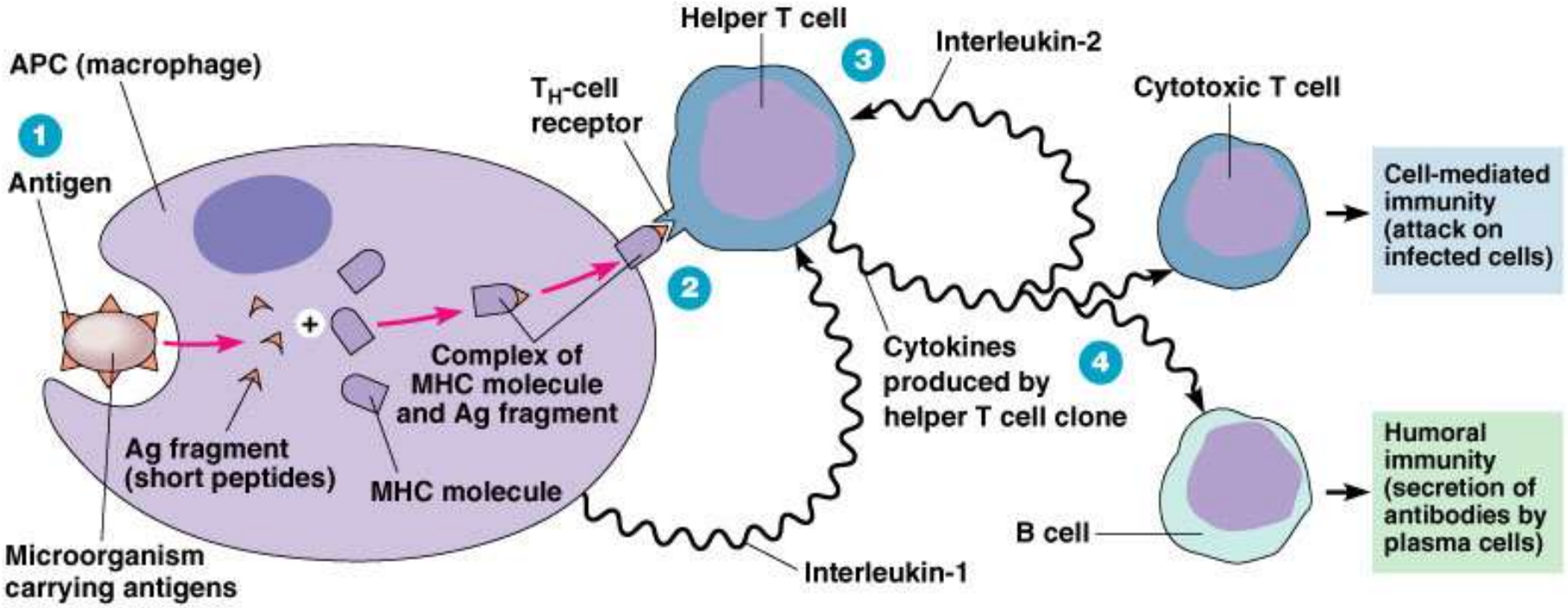
T Cells

- Helper T Cells (CD4, T_H)
  - T_H1 Activate cells related to cell-mediated immunity
  - T_H2 Activate B cells to produce eosinophils, IgM, and IgE
- Cytotoxic T Cells (CD8, T_C)
  - Destroy target cells with perforin

T Cells

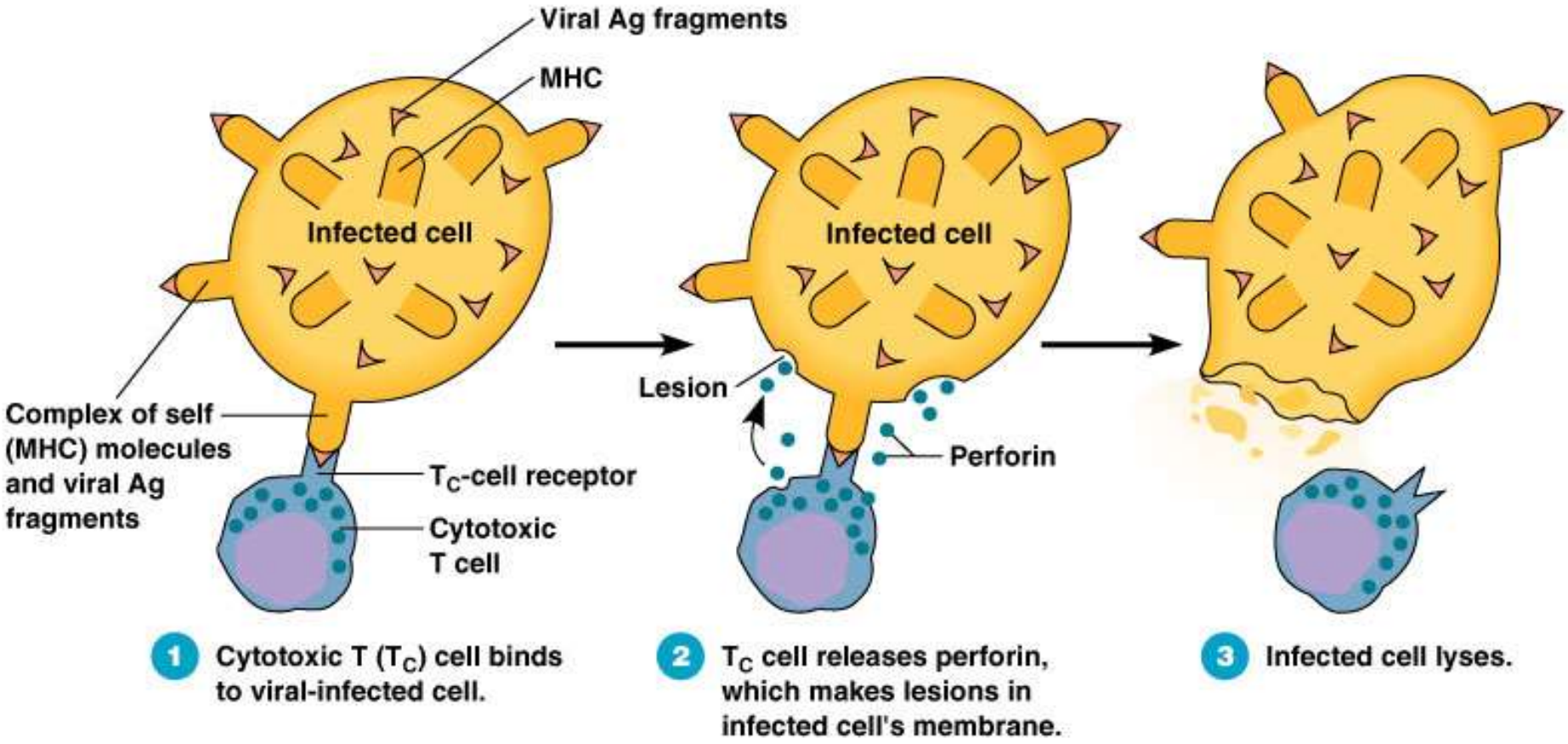
- Delayed Hypersensitivity T Cells (T_D)
  - Associated with allergic reaction, transplant rejection, and tuberculin skin test
- Suppressor T cells (T_S)
  - Turn off immune response when Ag no longer present

Helper T Cells



1. An antigen-presenting cell (APC) encounters and ingests a microoganism. Antigen fragments (short peptides) from the microorganism combine internally with MHC (self molecules) and the complex of MHC molecules and antigen fragments is presented on the surface of the APC.
2. A helper T (T_H) cell receptor binds to the complex, stimulating the APC to secrete interleukin-1.
3. This interleukin-1 stimulates the helper T cell to produce interleukin-2, which then stimulates that helper T cell to form a clone of helper T cells.
4. The cells of this clone in turn produce cytokines, stimulating cells of both immune systems.

Cell-mediated Cytotoxicity



General Microbiology

Laboratory Diagnosis

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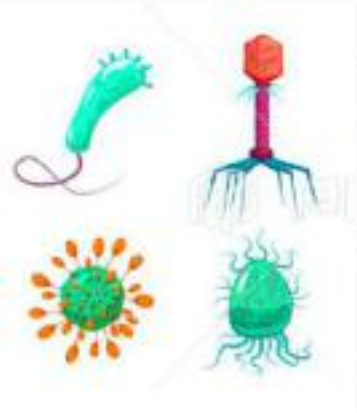
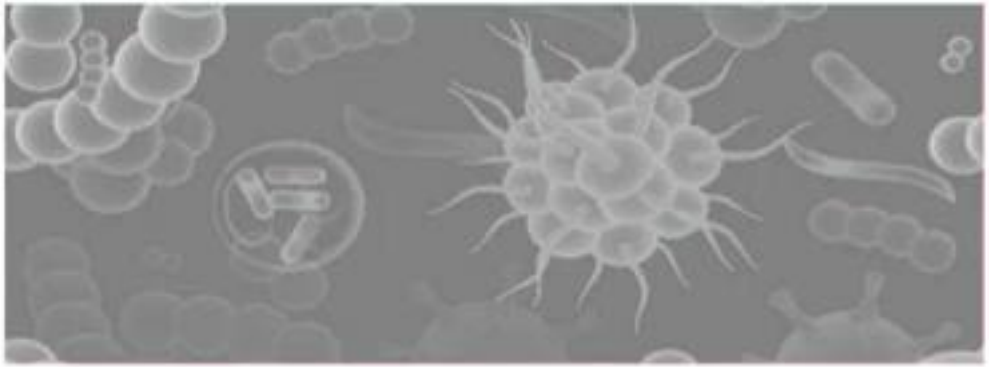
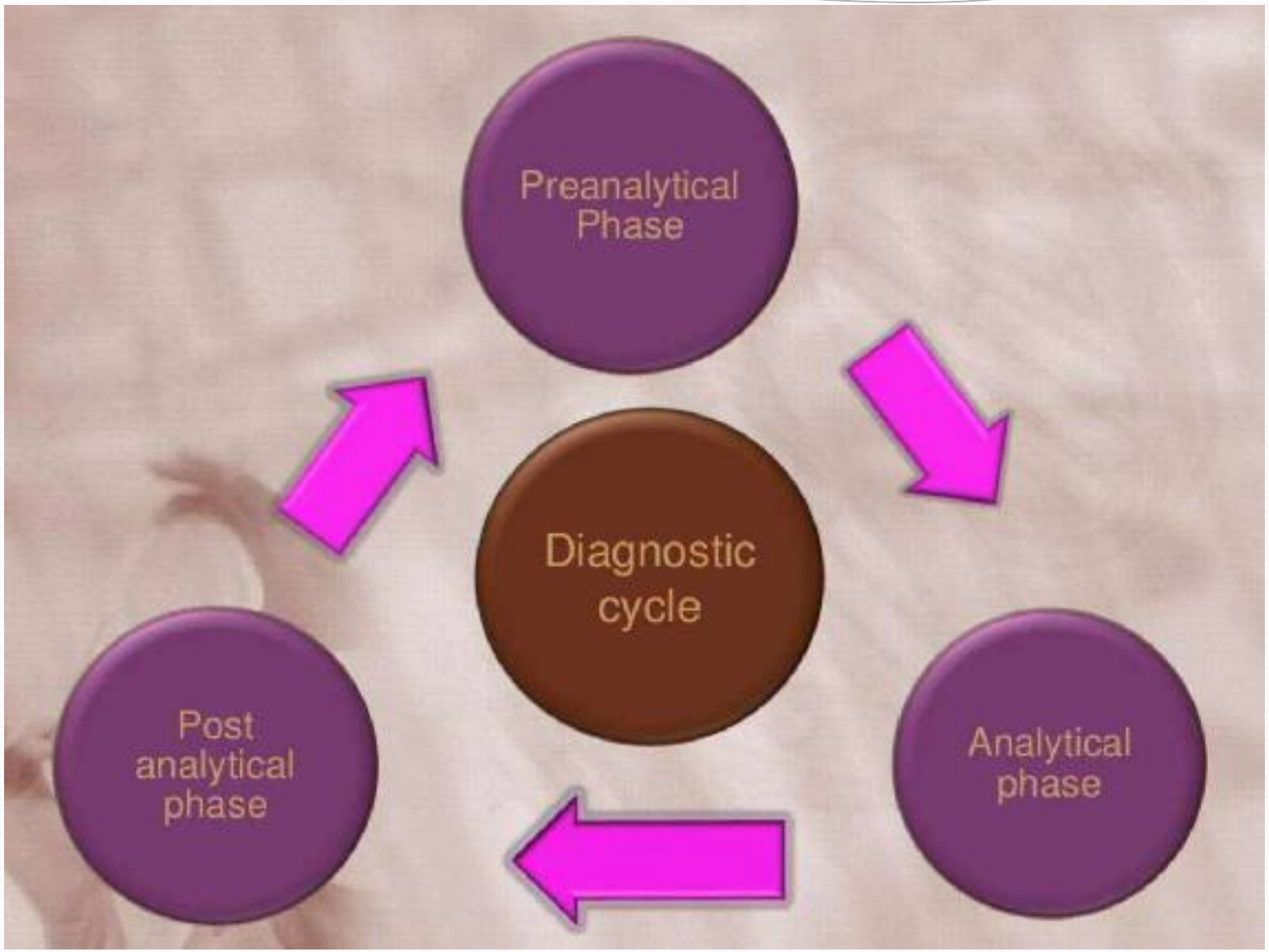
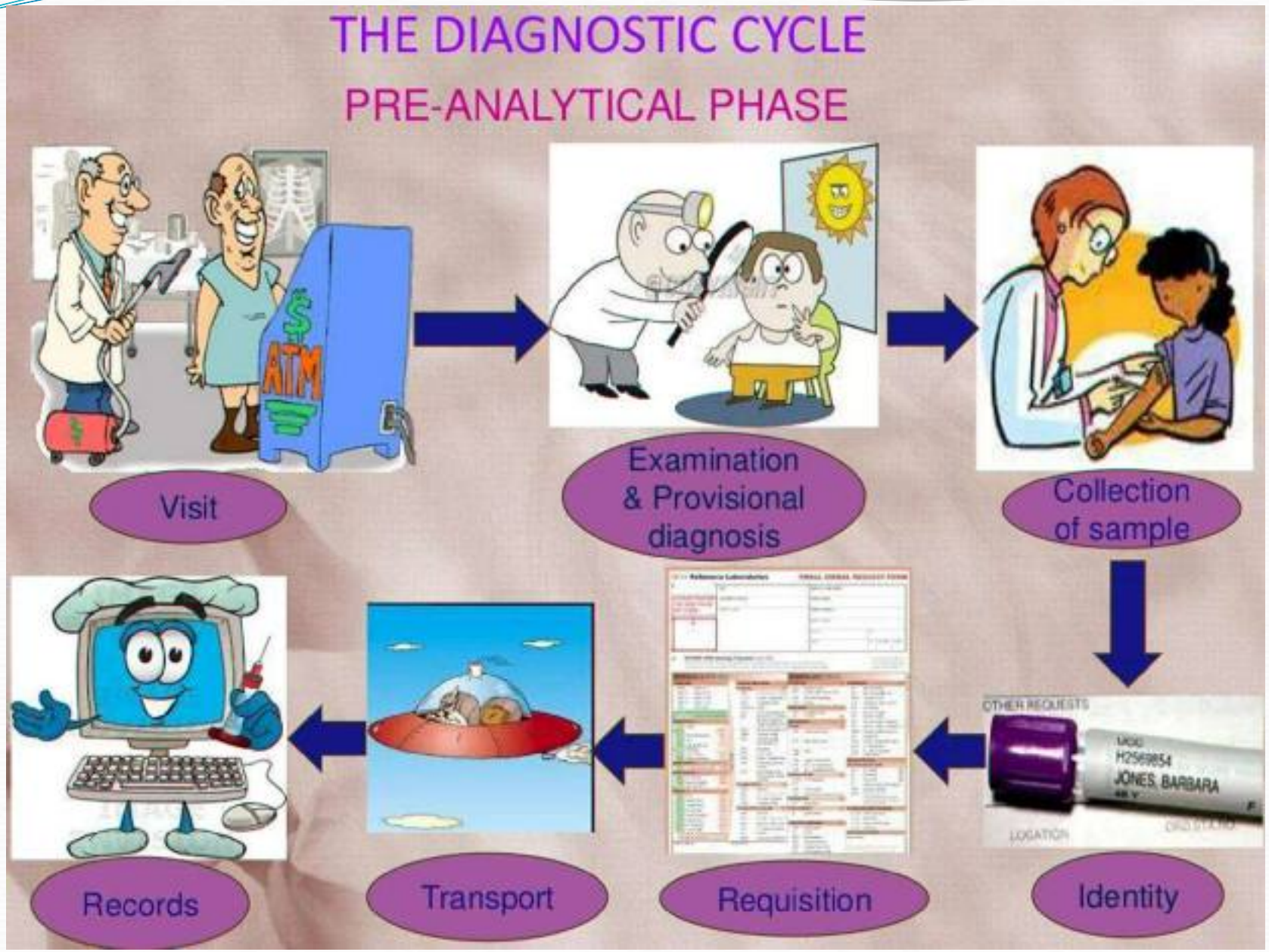


Diagnosis of Bacterial Infections

- The proper diagnosis of an infectious disease requires :
- (1) patient history.
- (2) physical examination of the patient.
- (3) carefully evaluating the patient's signs and symptoms.
- (4) conducting appropriate laboratory tests and other methods.
- (5) proper selection, collection, and transport of appropriate clinical specimens.




Preanalytical Phase
Diagnostic cycle
Analytical phase
Post analytical phase


THE DIAGNOSTIC CYCLE
PRE-ANALYTICAL PHASE
ATM
Visit
Examination & Provisional diagnosis
Collection of sample
OTHER REQUESTS
JONES, BARBARA
Identity
Requisition
Transport
Records

ANALYTICAL PHASE

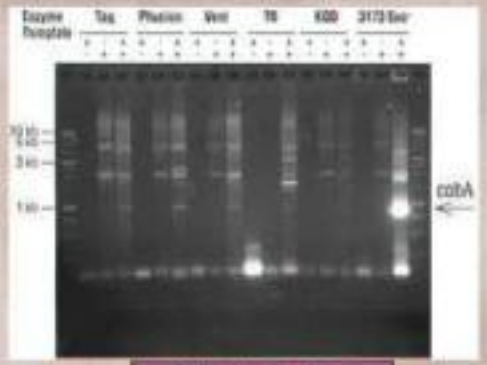
Macroscopy

Microscopy

Culture

Biochemical identification

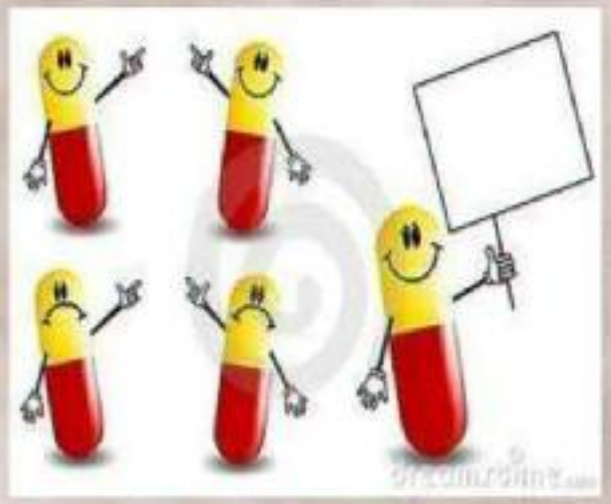
Serology



Molecular Analysis

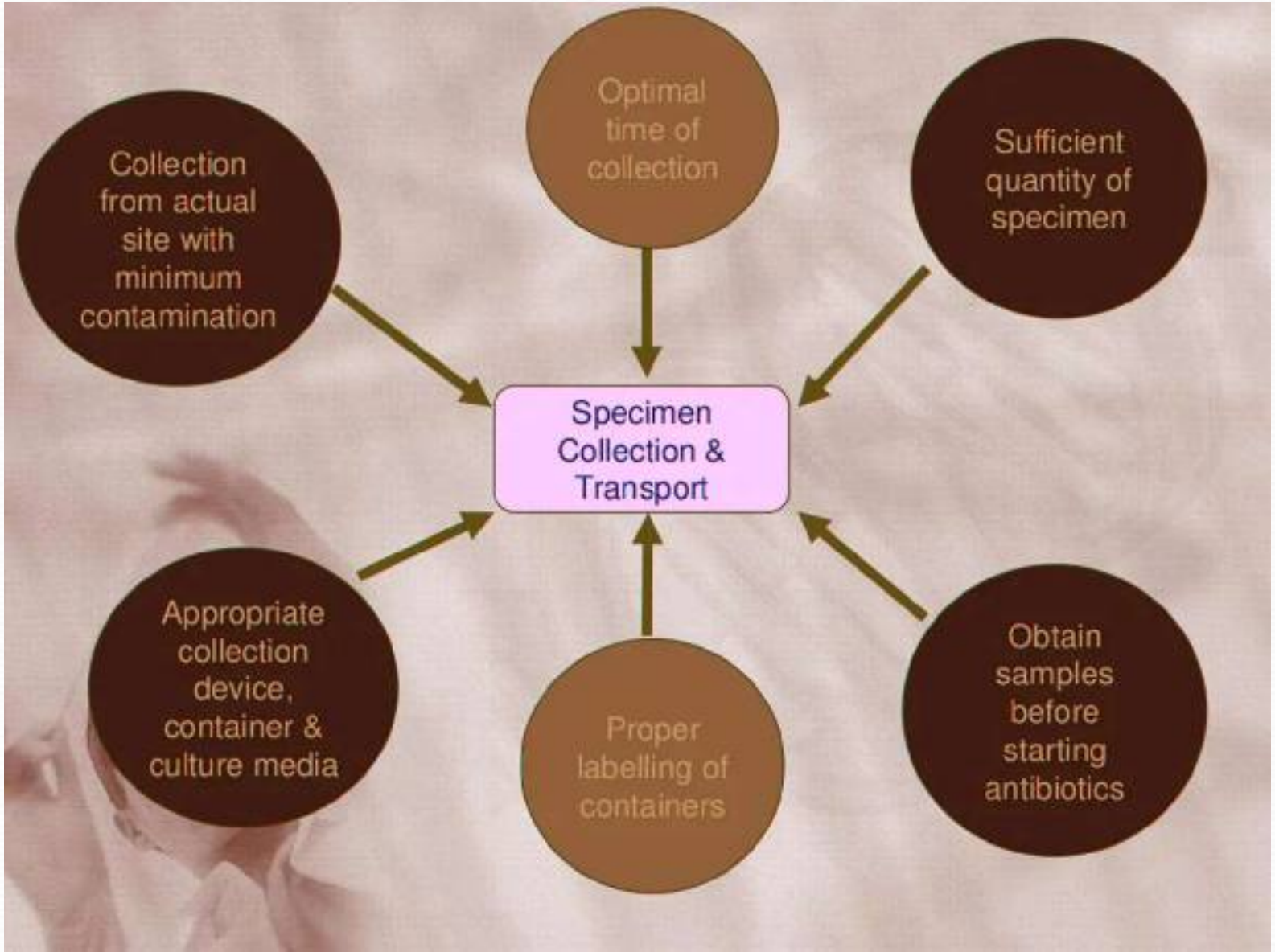
POST-ANALYTICAL PHASE

Reporting of Identification & Antibiotic sensitivity



Treatment

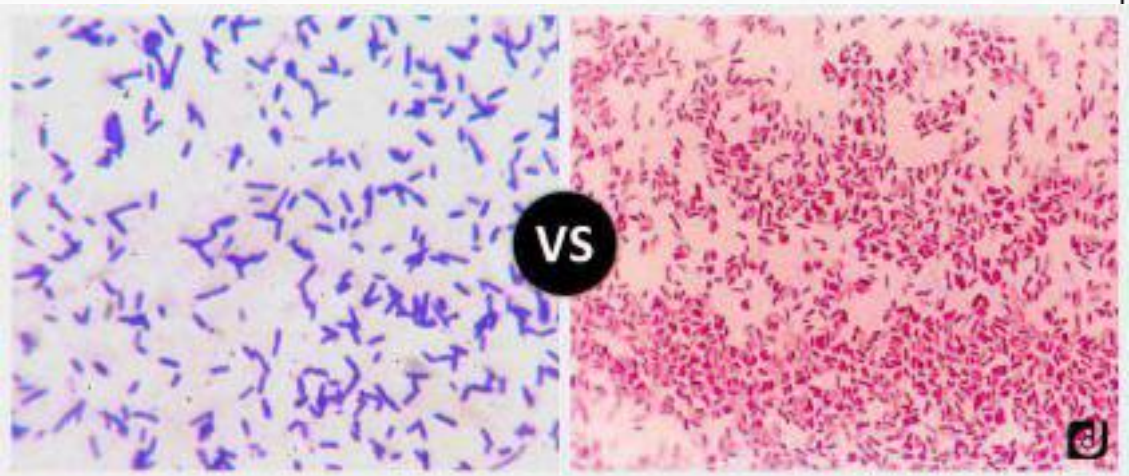
PRE-ANALYTICAL PHASE



Laboratory diagnosis of Bacterial Infections

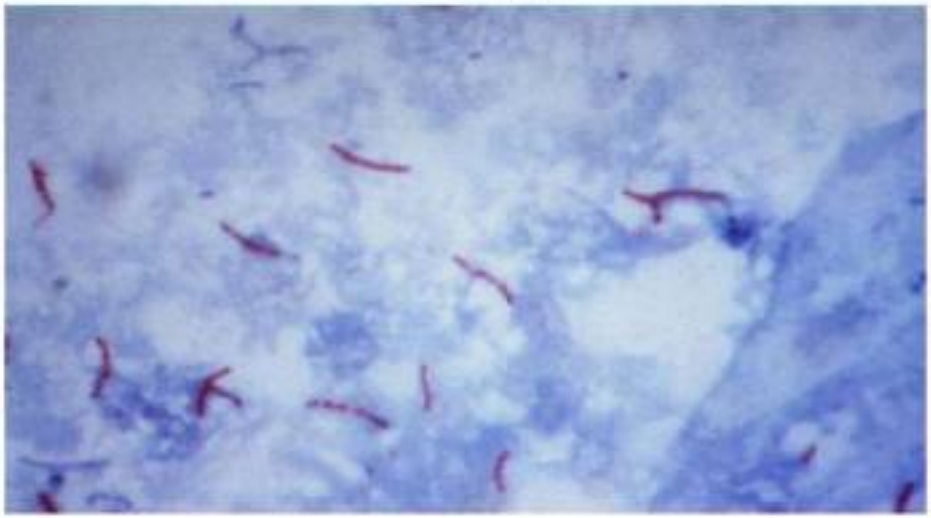
- 1- Microscopy
- 2- Culture techniques
- 3- Biochemical reactions
- 4- Serological identification:
- 5- Molecular biology techniques
- 6- Proteomic tests (MALDI-TOF-MS)

1- Microscopy



Gram Positive Bacteria vs. Gram Negative Bacteria

Mycobacterium tuberculosis - Ziehl-Neelsen Staining



2- Culture techniques

TABLE 9–3 Commonly Used Bacteriologic Agars and Their Function

Name of Agar[1]	Bacteria Isolated on the Agar	Function or Properties of the Agar
Blood	Various bacteria	Detect hemolysis
Bordet-Gengou	*Bordetella pertussis*	Increased concentration of blood allows growth
Charcoal-yeast extract	*Legionella pneumophila*	Increased concentration of iron and cysteine allows growth
Chocolate	*Neisseria meningitidis* and *Neisseria gonorrhoeae* from sterile sites	Heating the blood inactivates inhibitors of growth
Chocolate agar plus X and V factors	*Haemophilus influenzae*	X and V factors are required for growth
Egg yolk	*Clostridium perfringens*	Lecithinase produced by the organism degrades egg yolk to produce insoluble precipitate
Eosin-Methylene Blue	Various enteric gram-negative rods	Selects against gram-positive bacteria and differentiates between lactose fermenters and nonfermenters
Löwenstein-Jensen	*Mycobacterium tuberculosis*	Selects against gram-positive bacteria in respiratory tract flora and contains lipids required for growth
MacConkey	Various enteric gram-negative rods	Selects against gram-positive bacteria and differentiates between lactose fermenters and nonfermenters
Tellurite	*Corynebacterium diphtheriae*	Causes tellurite to become tellurium, which has black color
Thayer-Martin	*N. gonorrhoeae* from nonsterile sites	Chocolate agar with antibiotics to inhibit growth of normal flora
Triple sugar iron (TSI)	Various enteric gram-negative rods	Distinguishes lactose fermenters from nonfermenters and H_2S producers from nonproducers

[1]Names are listed in alphabetical order

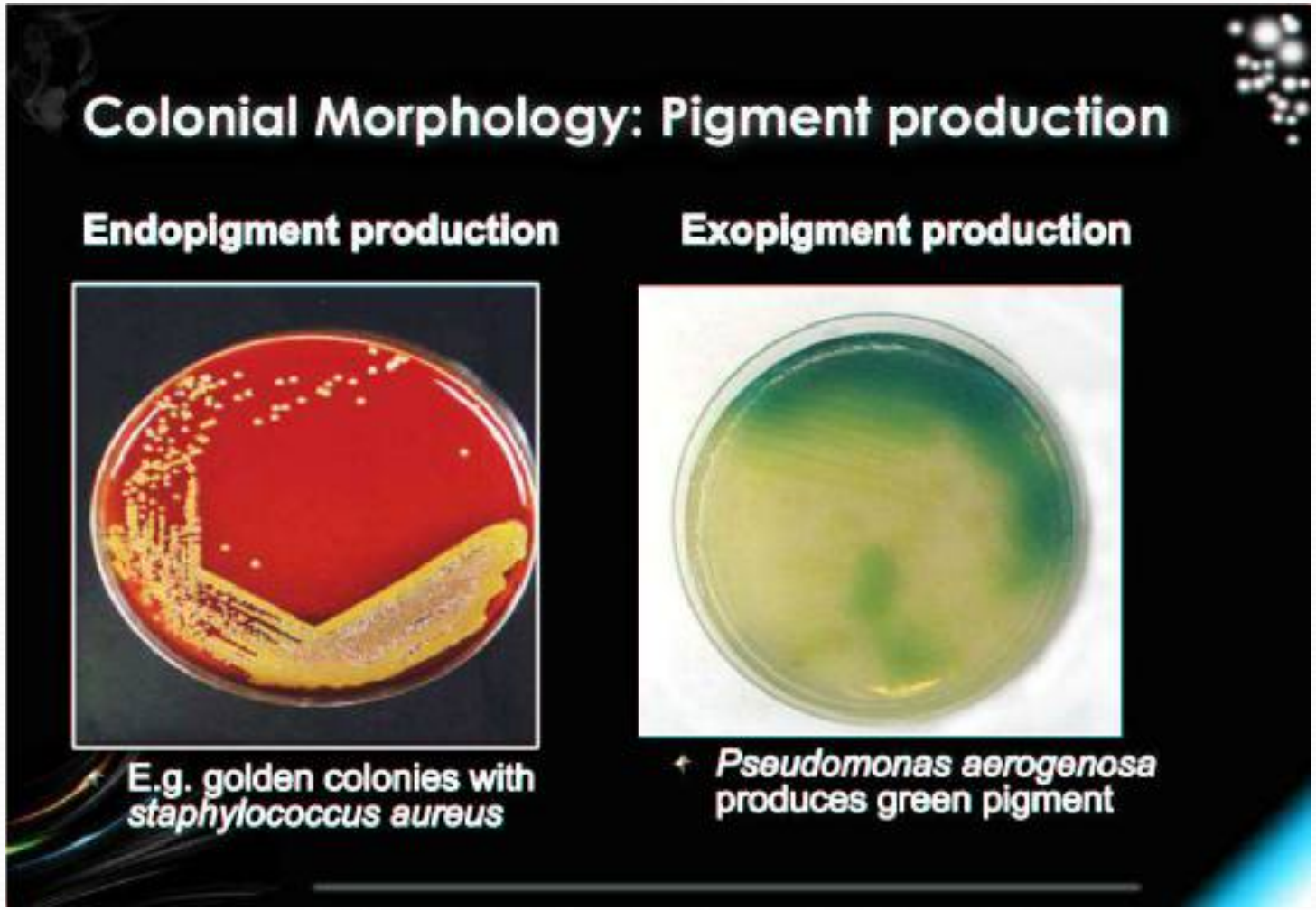
Colonial appearance on culture media

- * Colony morphology:
- . Shape . Size Edge of colony Color
- * Growth pattern in broth:
- . Uniform turbidity - . Sediment or surface pellicle
- * Pigment production:
- . Endopigment production (Staph. aureus)
- . Exopigment production (Ps. aeruginosa)
- * Haemolysis on blood agar:
- . Complete haemolysis (Strept. pyogenes)
- . Partial haemolysis (Strept. viridans)
- * Growth on MacConkey's medium:
- . Rose pink colonies (Lactose fermenters)
- . Pale yellow colonies (Non lactose fermenters)

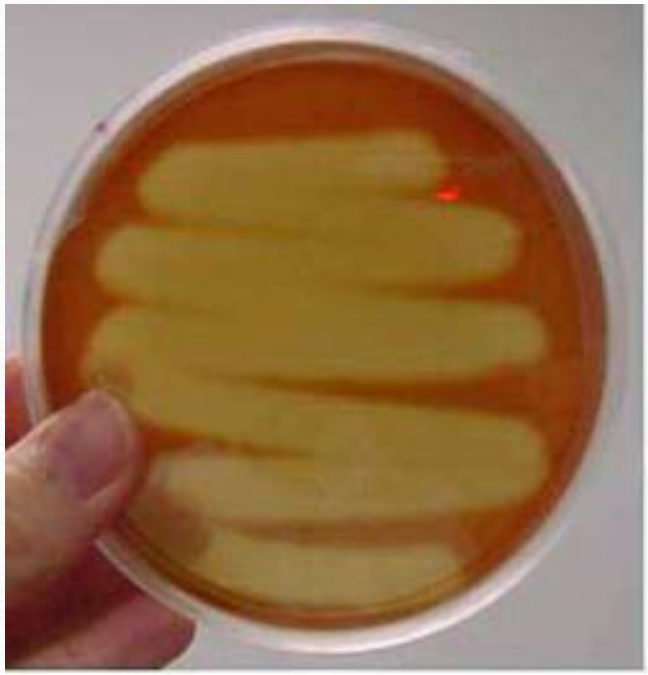
Pigment production:

1- Endopigment production

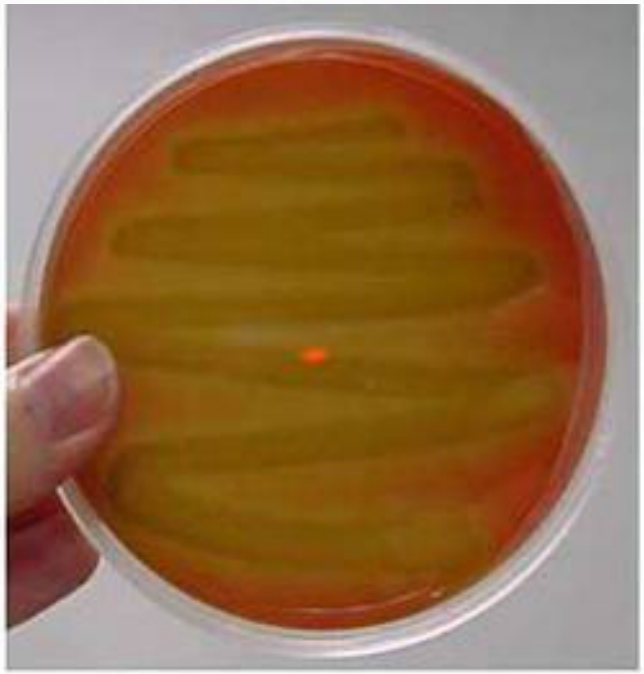
2- Exopigment production



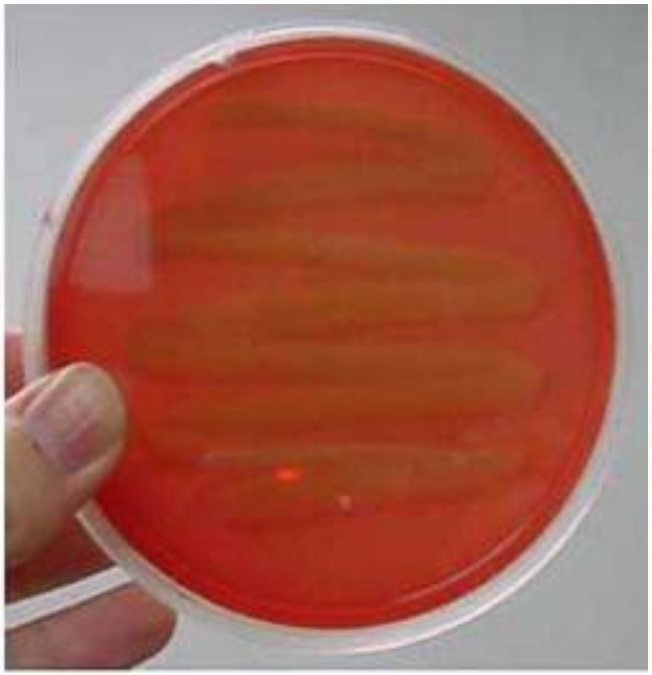
Effect on Blood agar:



Beta Hemolysis



Alpha Hemolysis



Gamma Hemolysis

Growth on MacConkey agar:

3- Biochemical reactions

- Use of substrates and sugars to identify pathogens:

a- Sugar fermentation:

- Organisms ferment sugar with production of acid only
- Organisms ferment sugar with production of acid and gas
- Organisms do not ferment sugar

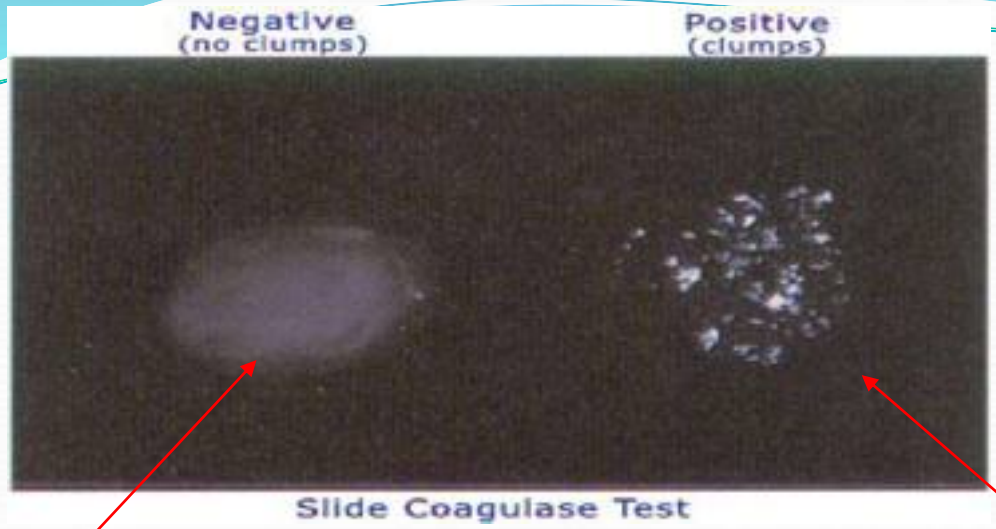
b- Production of indole:

- Depends on production of indole from amino acid tryptophan
- Indole is detected by addition of Kovac's reagent
- Appearance of red ring on the surface

c-Methyl red reaction (MR):

Fermentation of glucose with production of huge amount of acid

Lowering pH is detected by methyl red indicator



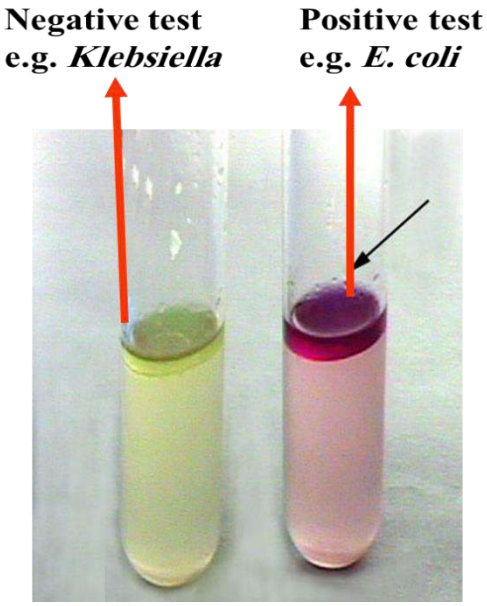
Streptococci

Catalase test

Staphylococci

S. aureus *S. epidermidis*

Coagulase test

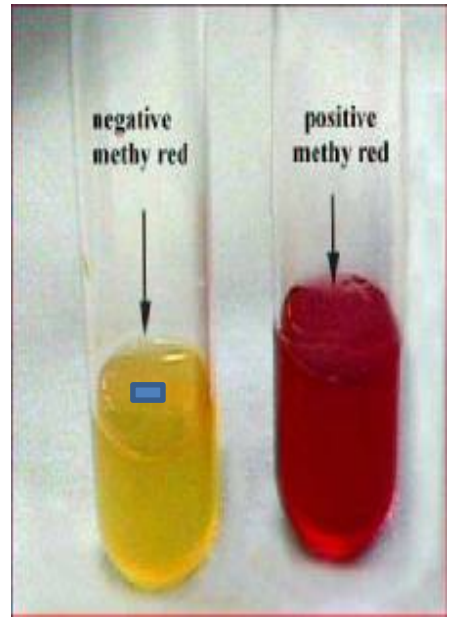


Indole test

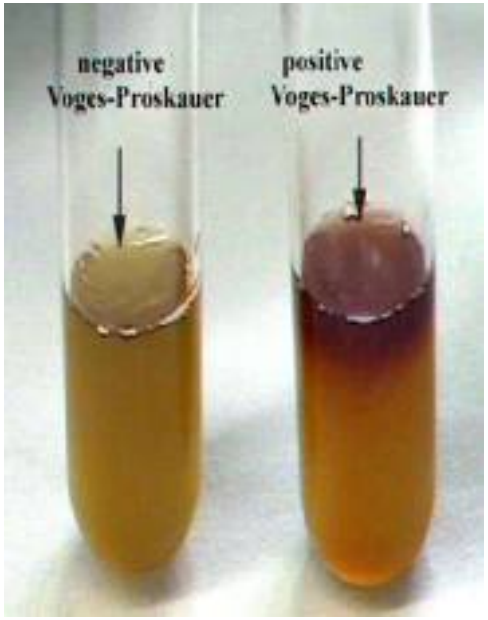
IMViC

E.Coli ++ --

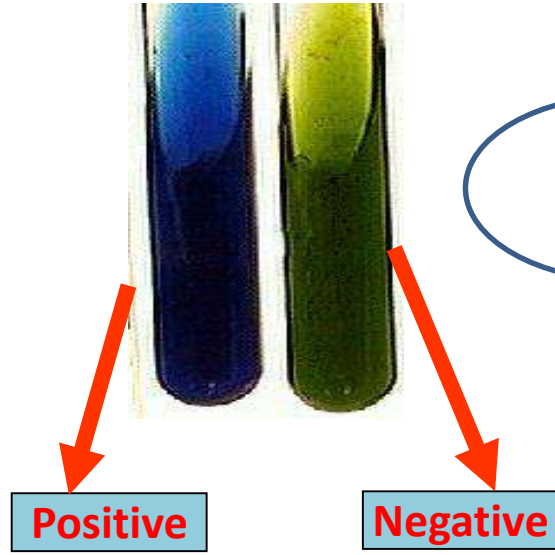
Klebsiella --++



Methyl red

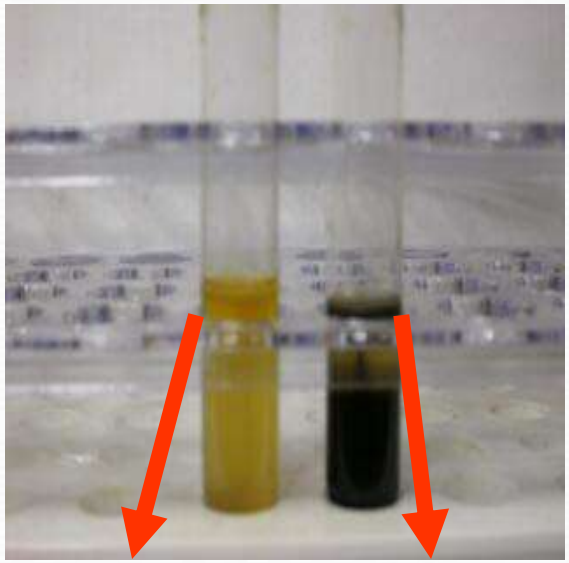


Voges Proskeur



Citrate

H2S production



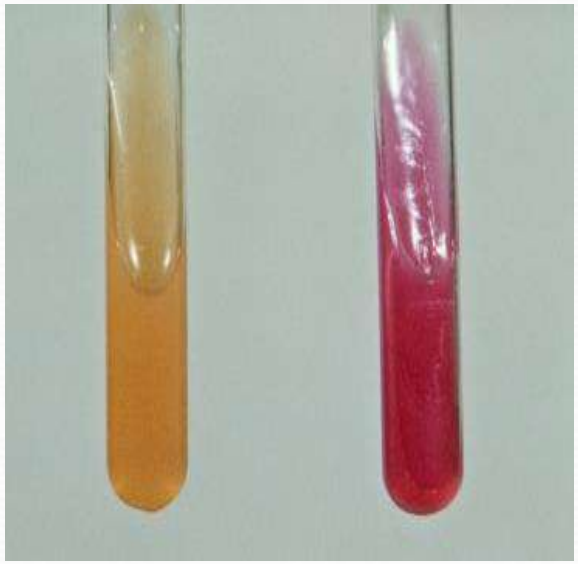
Negative

Positive

Shigella species

Salmonella species

Urease Test



Negative test

E. coli

Positive test

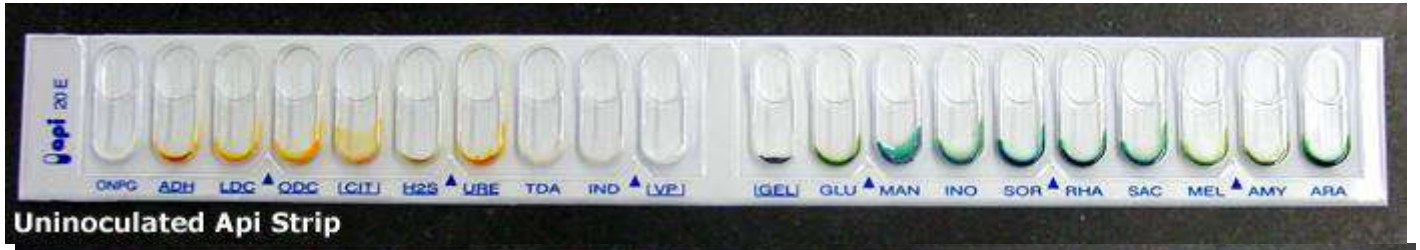
Proteus species

Oxidase test

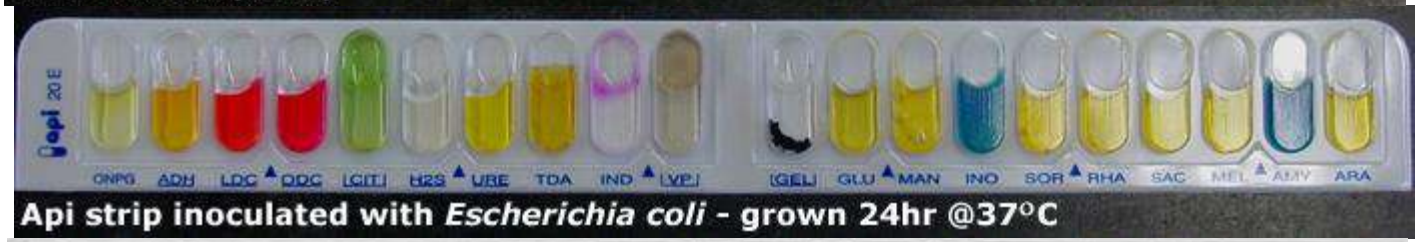
Pseudomonas

Vibrio

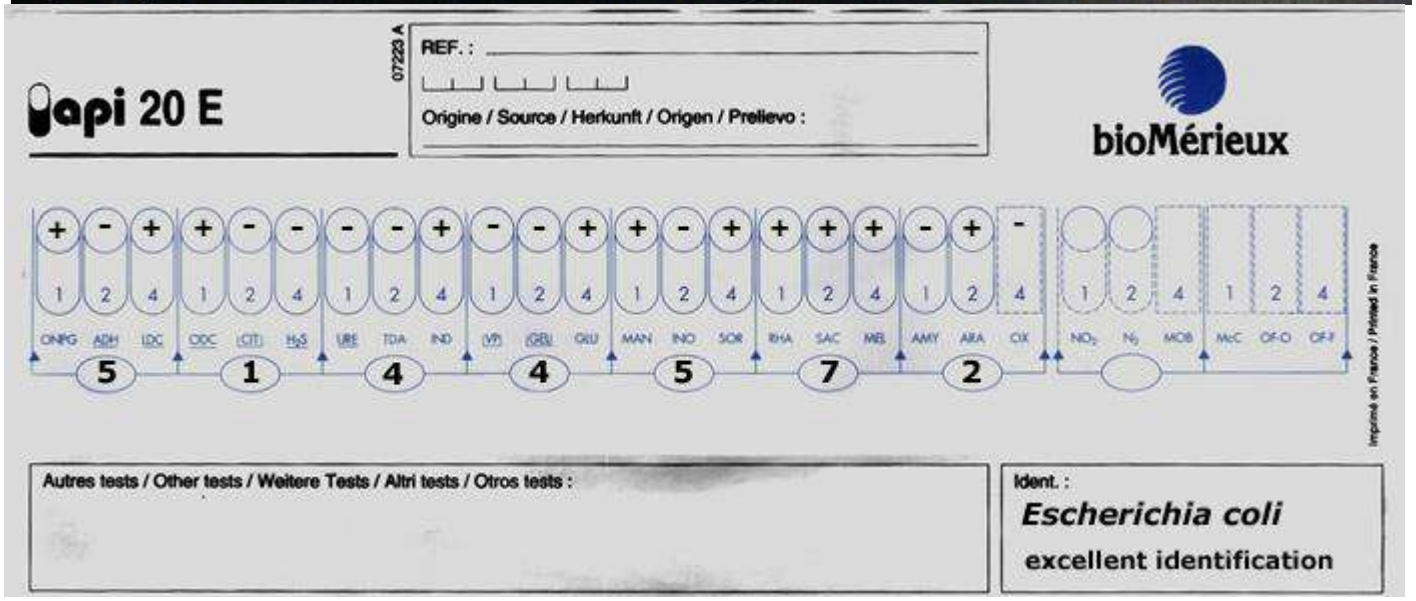
API System



Uninoculated Api Strip

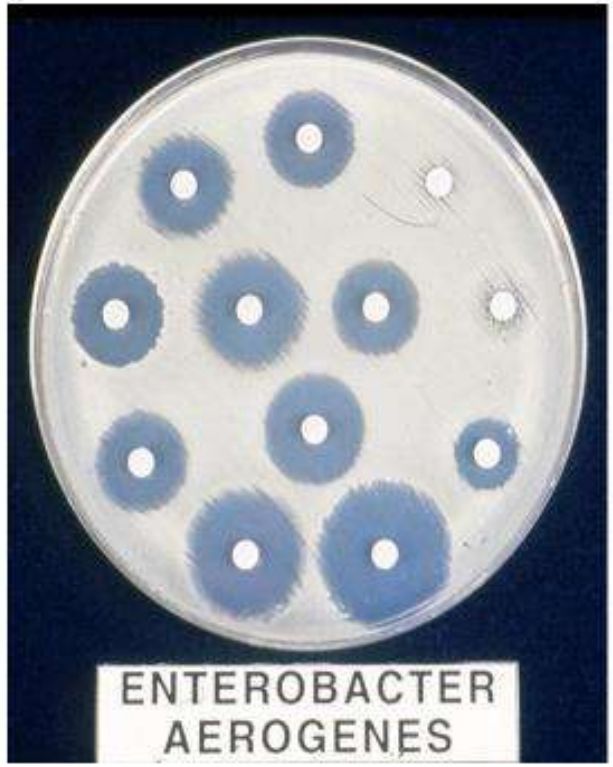


Api strip inoculated with *Escherichia coli* - grown 24hr @37°C



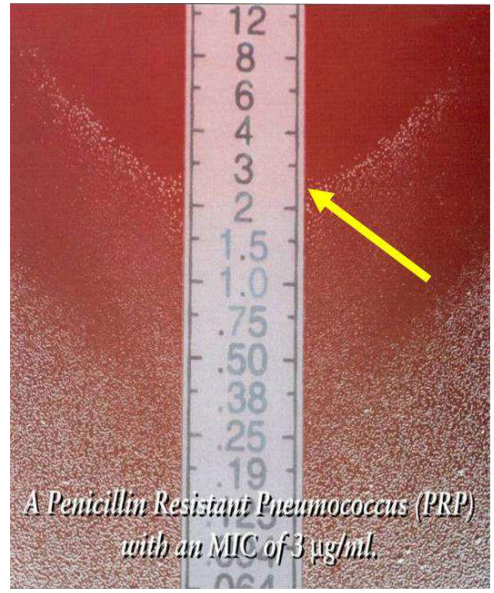
Antimicrobial Susceptibility Testing

Disk Diffusion Test

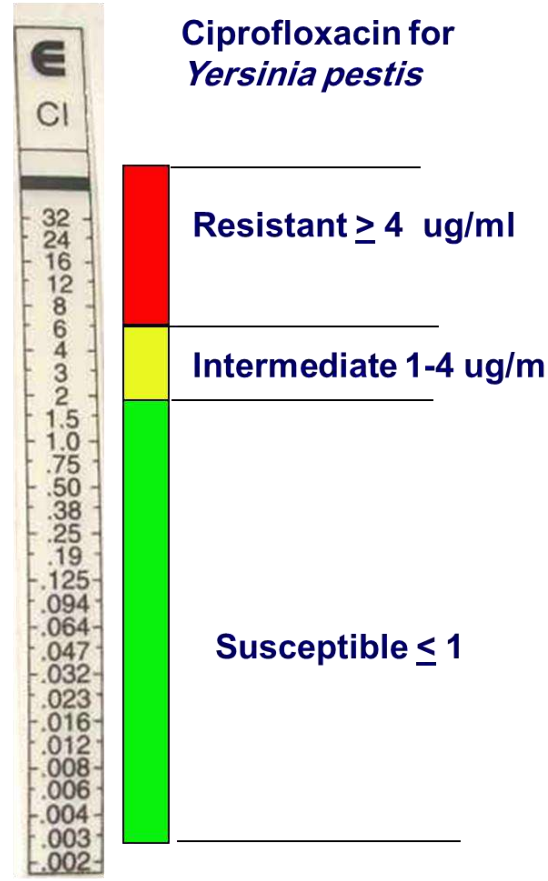


Minimal inhibitory concentration (MIC)

- The lowest concentration of antimicrobial agent that inhibits the growth of a bacterium
- Interpret:
  - Susceptible
  - Intermediate
  - Resistant



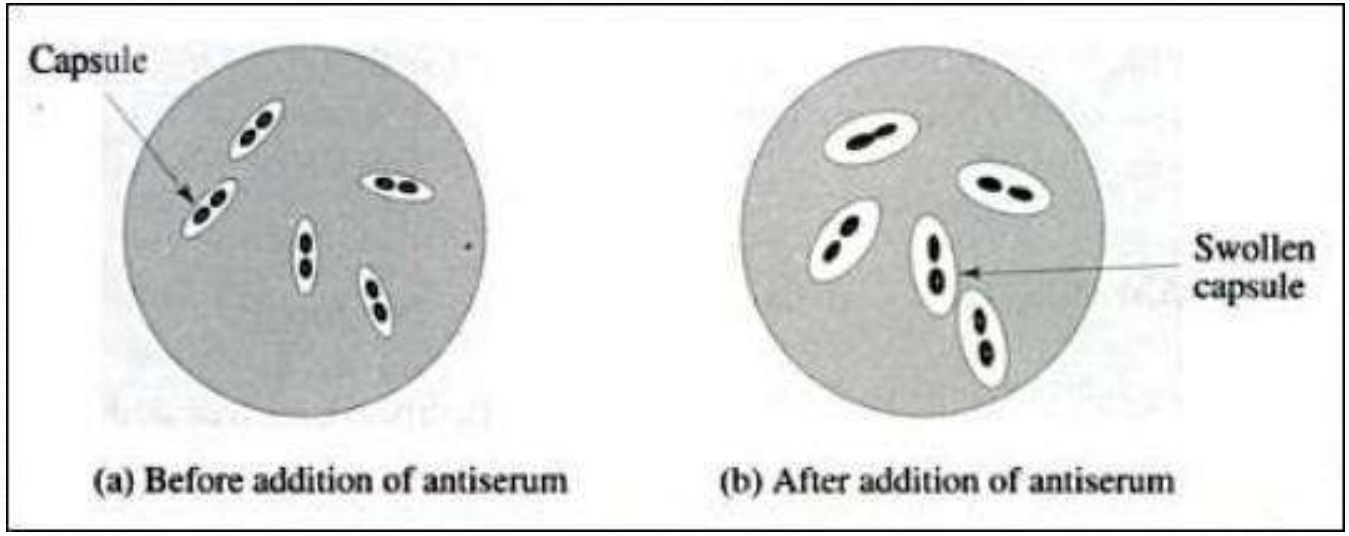
S. pneumoniae
Penicillin MIC = 3 µg/ml



4- Serological identification

Capsular Swelling (Quellung) Reaction

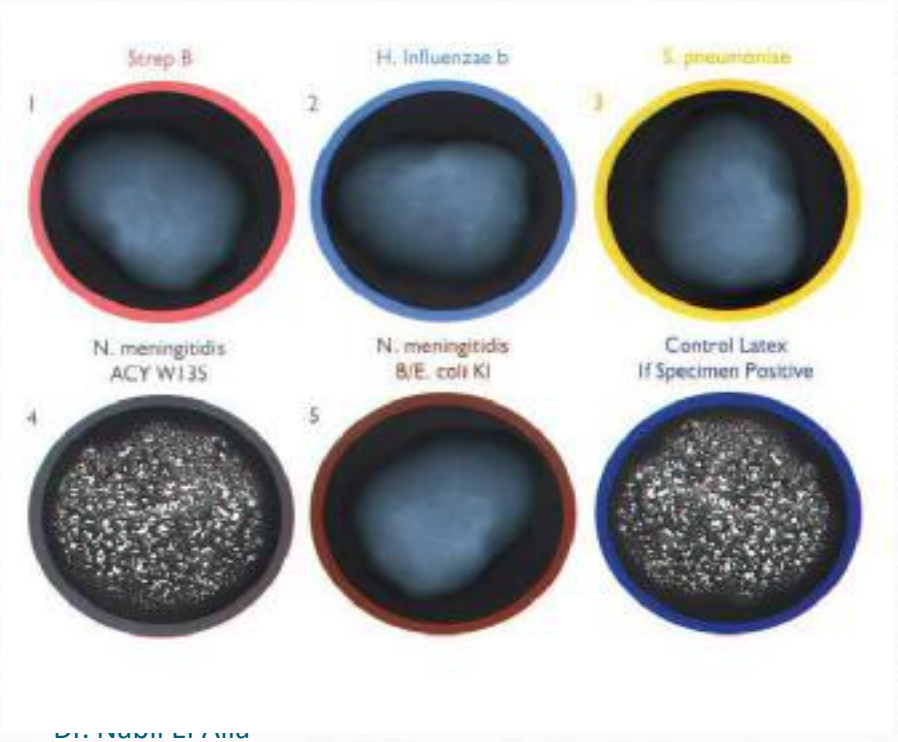
- Based on the microscopic observation that the capsule swells in the presence of homologous antiserum.
- Antisera against the following organisms are available: all serotypes of S. pneumoniae (Omniserum), H. influenzae type b, and N. meningitides groups A and C.



Slide Agglutination Test

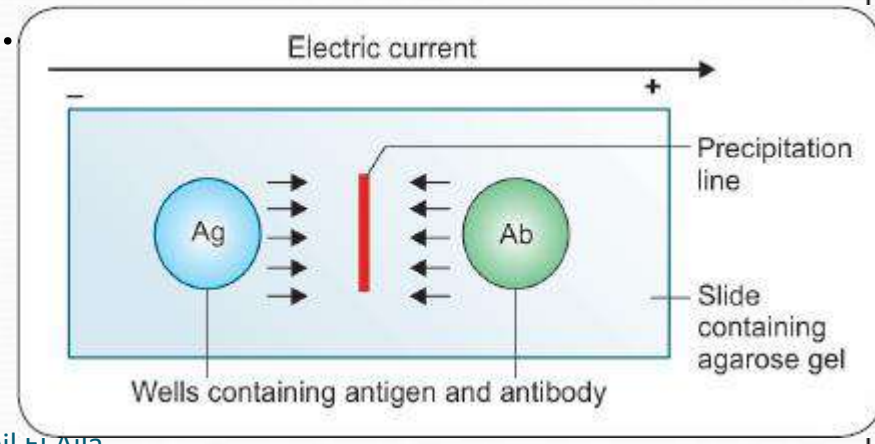
- Antisera can be used to identify *Salmonella* and *Shigella* by causing agglutination (clumping) of the unknown organism.
- Antisera directed against the cell wall O antigens of *Salmonella* and *Shigella* are commonly used in hospital laboratories.
- Antisera against the flagellar H antigens and the capsular Vi antigen of *Salmonella* are used in public health laboratories for epidemiologic purposes.

- **Latex Agglutination Test**
- Latex beads coated with specific antibody are agglutinated in the presence of the homologous bacteria or antigen.
- This test is used to determine the presence of the capsular antigen of H. influenzae, N. meningitidis, several species of streptococci, and the yeast C. neoformans.



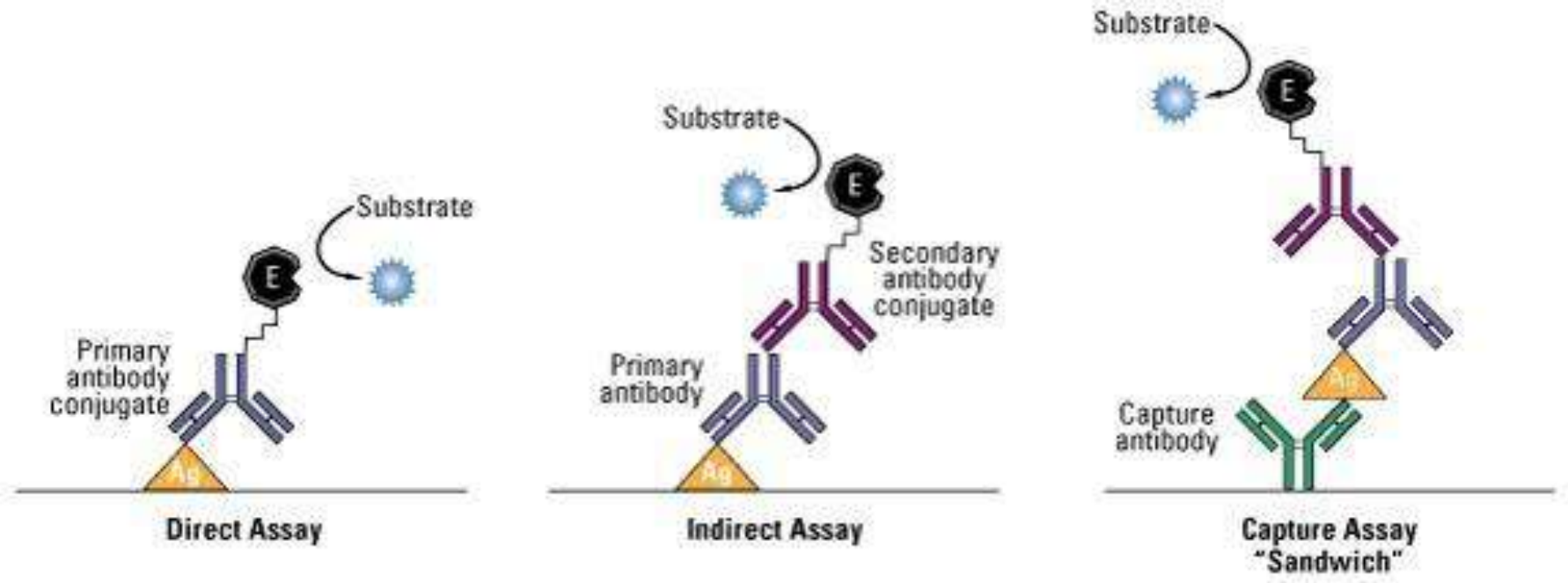
Counterimmunoelectrophoresis Test:

- In this test, the unknown bacterial antigen and a known specific antibody move toward each other in an electrical field.
- If they are homologous, a precipitate forms within the agar matrix.
- Because antibodies are positively charged at the pH of the test, only negatively charged antigens, usually capsular polysaccharides, can be assayed.
- The test can be used to detect the presence in the spinal fluid of the capsular antigens of H. influenzae, N. meningitidis, S. pneumoniae, and group B streptococci.



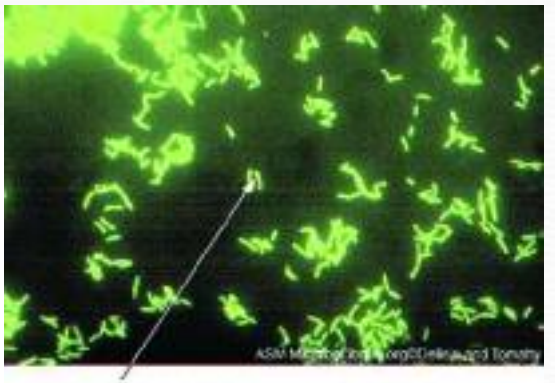
Enzyme-Linked Immunosorbent Assay

- In this test, a specific antibody to which an easily assayed enzyme has been linked is used to detect the presence of the homologous antigen.
- This test is useful in detecting a wide variety of bacterial, viral, and fungal infections.

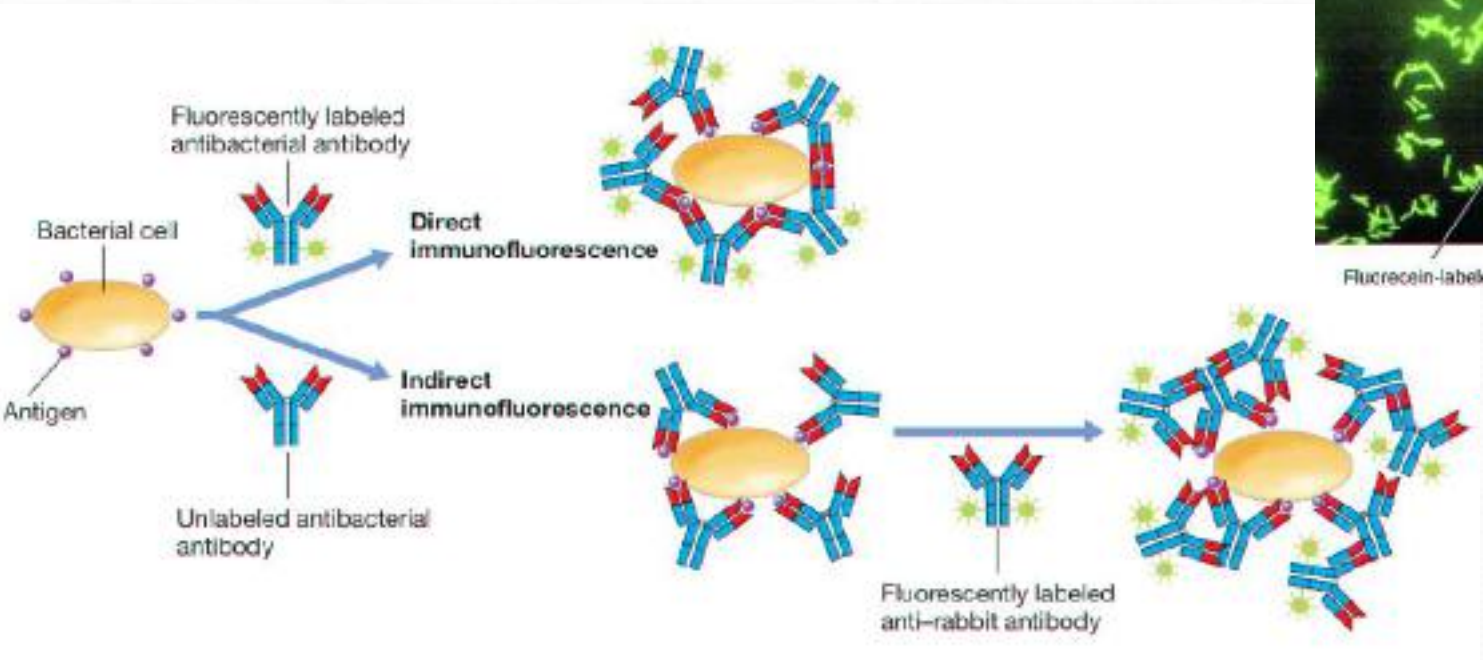


Fluorescent Antibody Tests

- A variety of bacteria can be identified by exposure to known antibody labeled with fluorescent dye, which is detected visually in the ultraviolet microscope.
- Various methods can be used, such as the direct and indirect techniques



Fluorescein-labeled antibody attached to *Legionella* bacilli

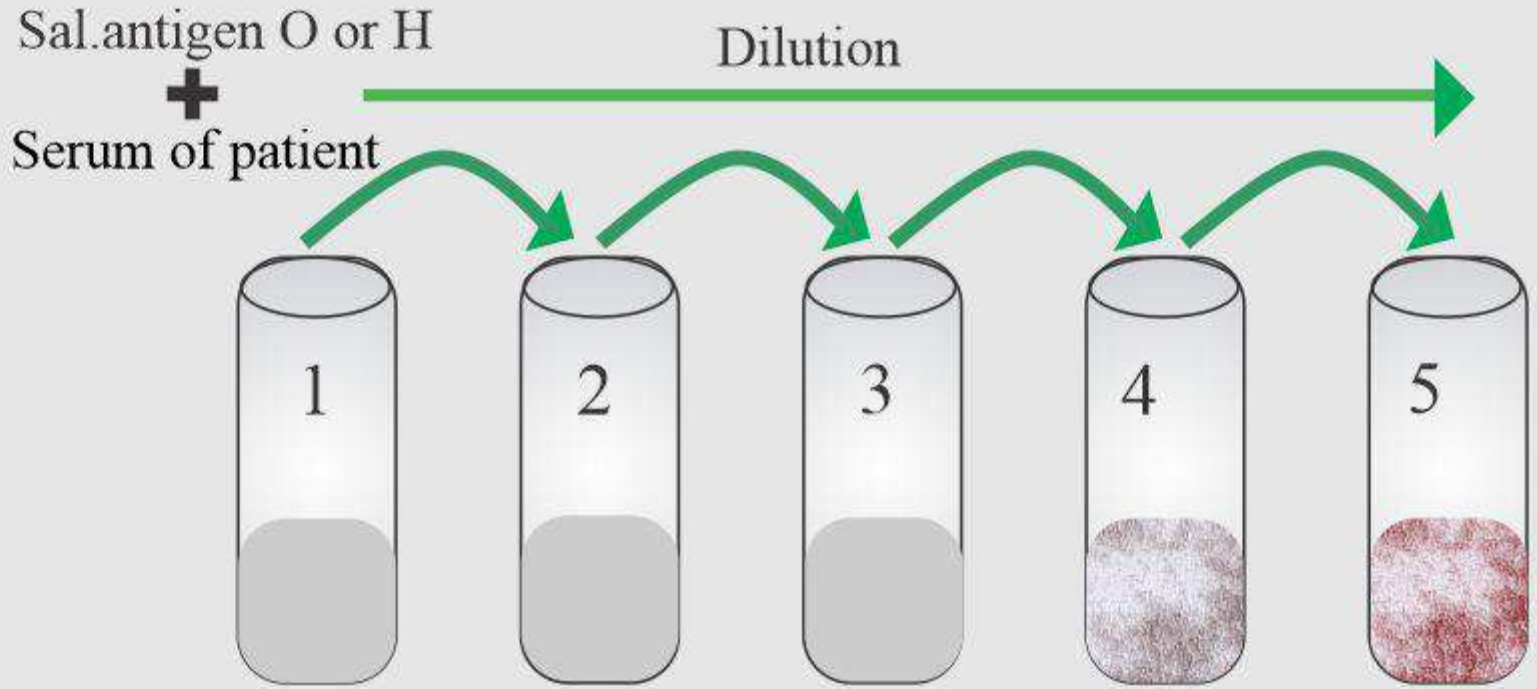


Identification of Serum Antibodies with Known Antigens

Slide or Tube Agglutination Test

- In this test, serial twofold dilutions of a sample of the patient's serum are mixed with standard bacterial suspensions.
- The highest dilution of serum capable of agglutinating the bacteria is the titer of the antibody.
- As with most tests of a patient's antibody, at least a fourfold rise in titer between the early and late samples must be demonstrated for a diagnosis to be made.
- This test is used primarily to aid in the **diagnosis of typhoid fever, brucellosis, tularemia, plague, leptospirosis, and rickettsial diseases.**

Widal test



	1	2	3	4	5
Dilution →	1:20	1:40	1:80	1:160	1:320
Agglutination →	Negative	Negative	Negative	Positive ++	Positive ++ ++

Start → Titer = 1:160 (End point)

Serologic Tests for Syphilis

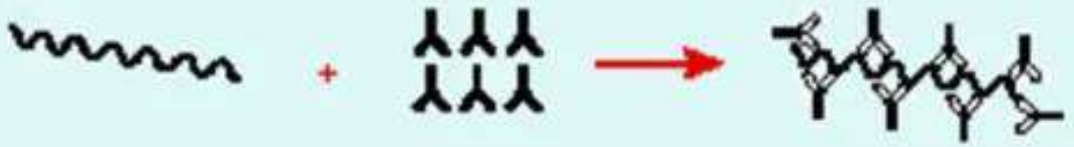
- The detection of antibody in the patient's serum is frequently used to diagnose syphilis, because T. pallidum does not grow on laboratory media. There are two kinds of tests.
- **(1) The nontreponemal tests** use a cardiolipin–lecithin–cholesterol mixture as the antigen, not an antigen of the organism.
- Cardiolipin (diphosphatidylglycerol) is a lipid extracted from normal beef heart.
- Flocculation (clumping) of the cardiolipin occurs in the presence of antibody to T. pallidum.
- The VDRL and RPR tests are nontreponemal tests commonly used as screening procedures.
- They are not specific for syphilis but are inexpensive and easy to perform.

(2) The treponemal tests use T. pallidum as the antigen.

- The two most widely used treponemal tests are the Fluorescent treponemal antibody-absorption (FTA-ABS) test and the MHA-TP (microhemagglutination–Treponema pallidum) tests.
- In the FTA-ABS test, the patient's serum sample, which has been absorbed with treponemes other than T. pallidum to remove nonspecific antibodies, is reacted with nonviable T. pallidum on a slide.
- Fluorescein labeled antibody against human immunoglobulin G (IgG) is then used to determine whether IgG antibody against
- T. pallidum is bound to the organism.

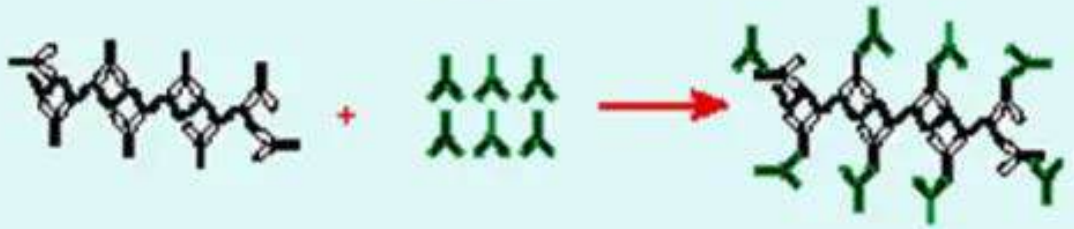
Step 1

Antigen	Patient's specimen containing antibody (diluted 1 :5 in sorbent)	Antigen-antibody complex
Treponema pallidum		



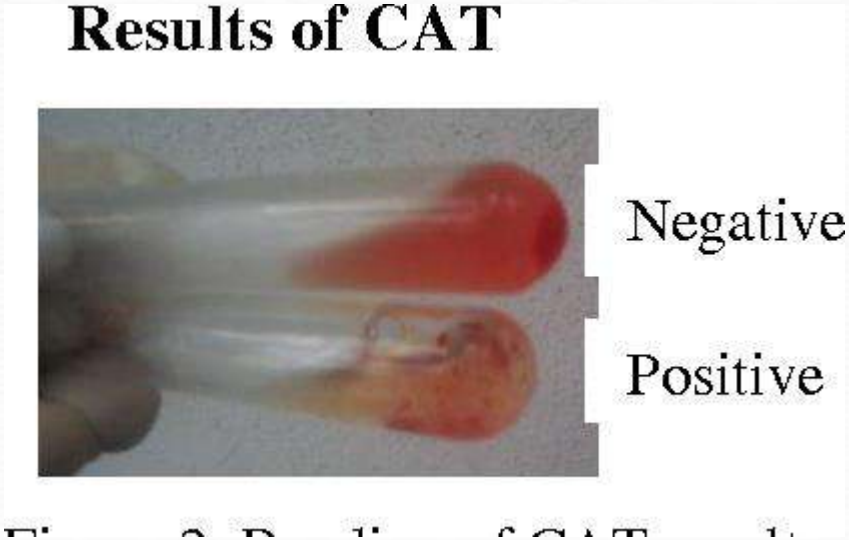
Step 2

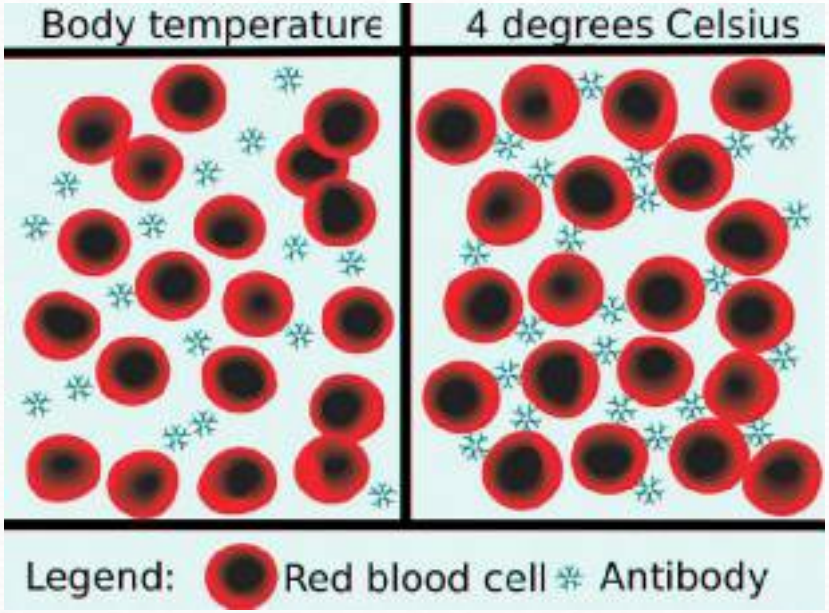
The treponeme is coated with antibody. This coating can be "stained" with a conjugate.	Conjugate (Fluorescein-conjugated animal antiserum to human globulin)	Fluorescence (2nd antigen-antibody complex)



Cold Agglutinin Test

- Patients with Mycoplasma pneumoniae infections develop autoimmune antibodies that agglutinate human red blood cells in the cold (4°C) but not at 37°C.
- These antibodies occur in certain diseases other than Mycoplasma infections; thus false-positive results can occur.





5- Molecular biology techniques

- There are three types of **nucleic acid–based tests** used in the diagnosis of bacterial diseases:
  - Nucleic acid amplification tests,
  - Nucleic acid probes,
  - Nucleic acid sequence analysis.
- Nucleic acid–based tests are highly specific, quite sensitive (especially the amplification tests), and much faster than culturing the organism.
- These tests are especially useful for those bacteria that are difficult to culture, such as Chlamydia and Mycobacterium species.

- Nucleic acid amplification tests (NAAT) utilize **the polymerase chain reaction (PCR)** or other amplifying process to increase the number of bacteria-specific DNA or RNA molecules so the sensitivity of the test is significantly higher than that of unamplified tests.
- Many bacteria can be identified using these tests, but they are especially useful in detecting C. trachomatis and N. gonorrhoeae in urine samples in sexually transmitted disease (STD) clinics.
- These tests are also used to identify M. tuberculosis in sputum samples.

❖ **PCR:** A process which “Amplifies” or “Copies” a piece of DNA repeatedly until there is an amount which is great enough to observe visually.

❖ It is an in vitro DNA amplification process which can rapidy (i.e in few hours) amplify a selected region of DNA up to million fold

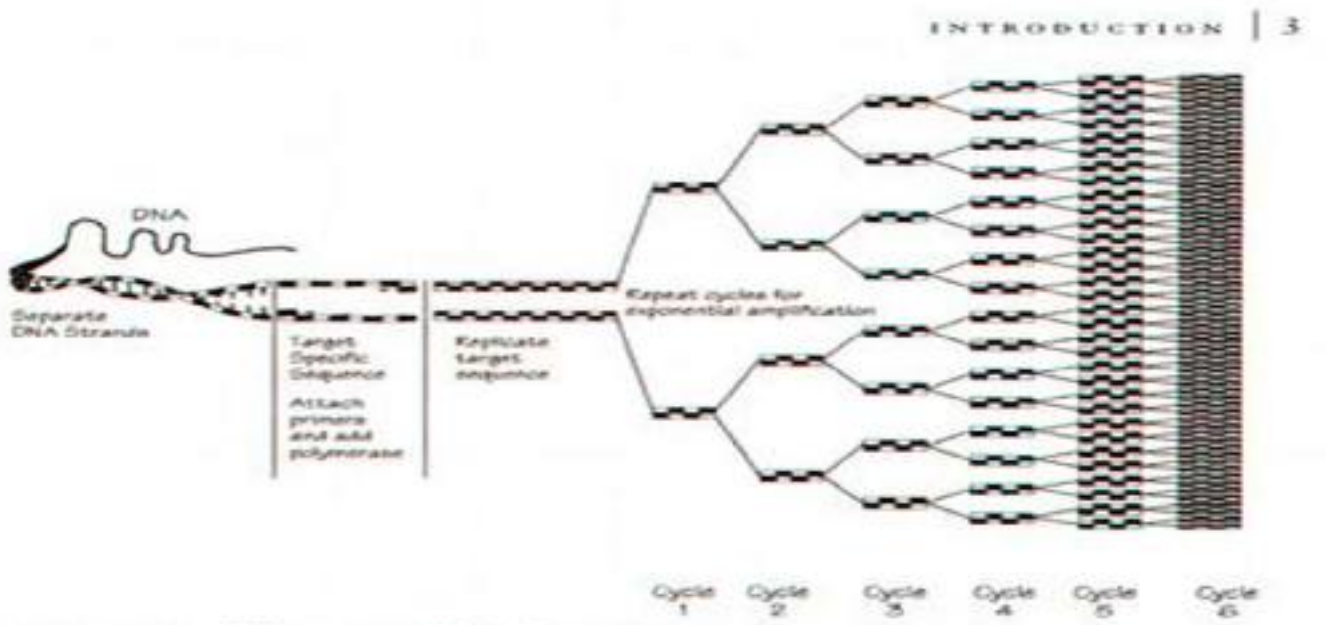
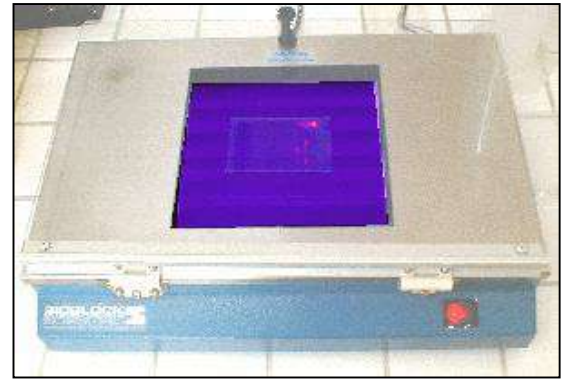


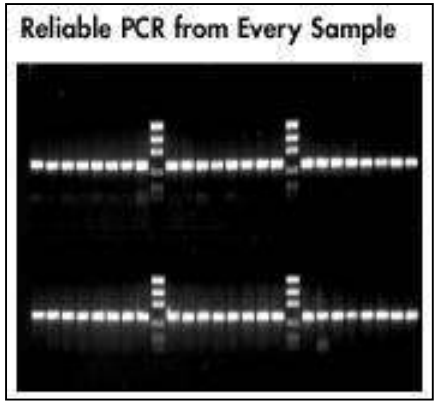
FIGURE 1. Polymerase Chain Reaction

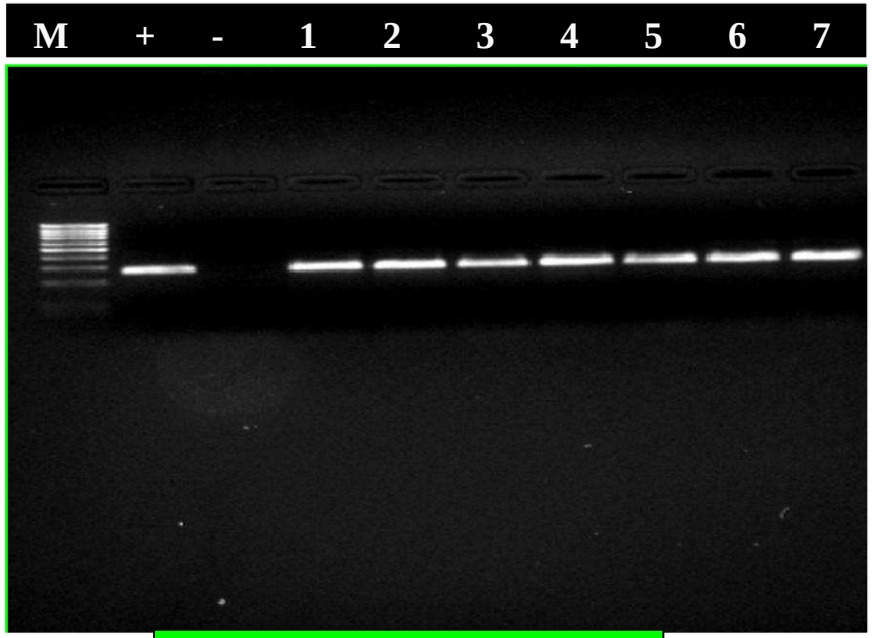
PCR

3-4 hours



UV visualisation

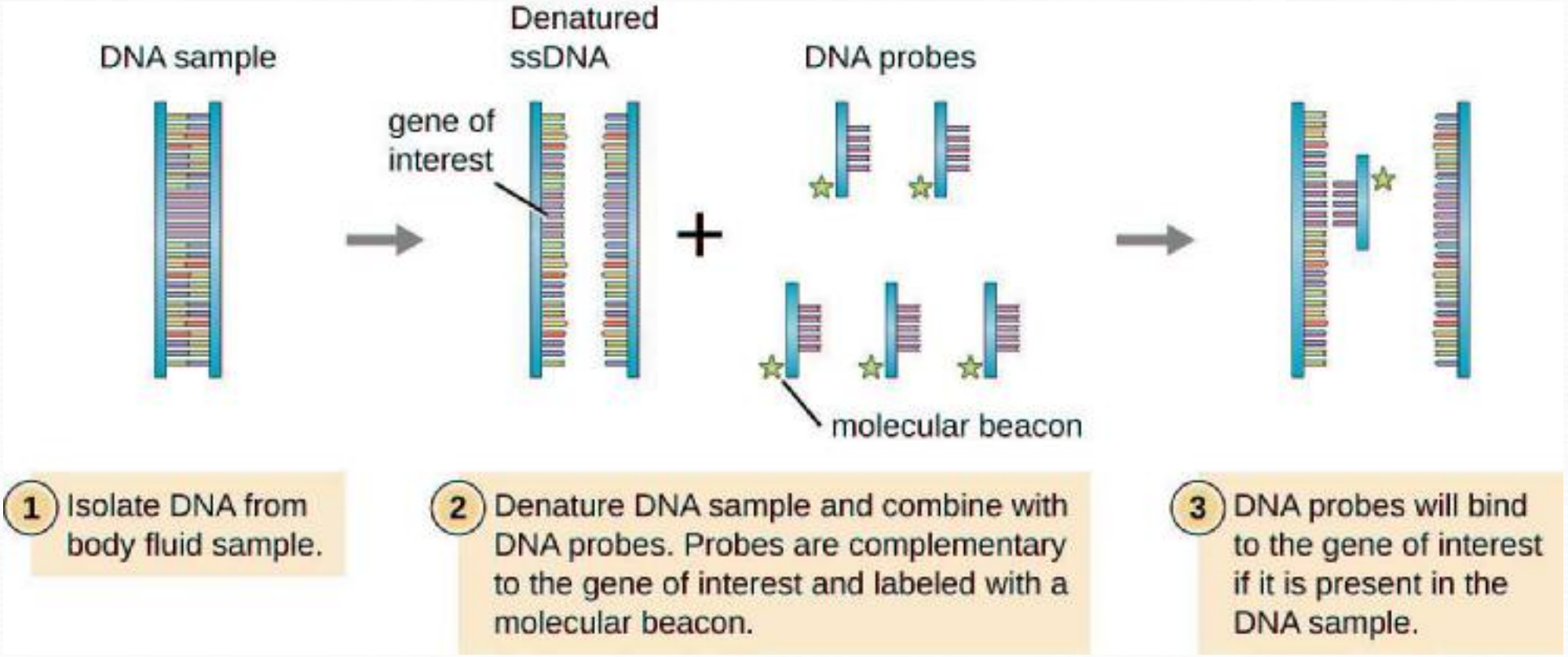




16S rRNA - PCR

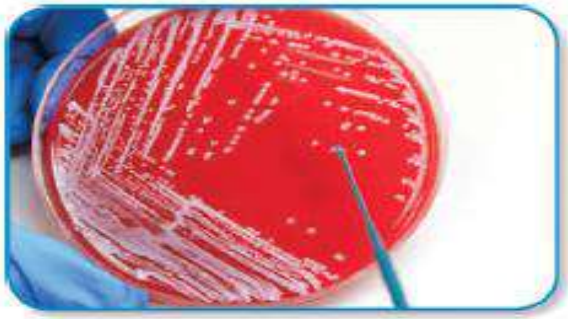
IS*6110*-PCR

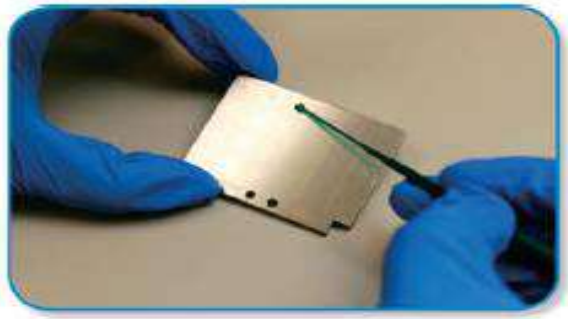
- **Tests that use nucleic acid probes** are designed to detect bacterial DNA or RNA directly (without amplification) using a labeled DNA or RNA probe that will hybridize specifically to the bacterial nucleic acid.
- These tests are simpler to perform than the amplification tests but are less sensitive.
- **Nucleic acid sequence** analysis is used to identify bacteria based on the base sequence of the organism's ribosomal RNA.
- An organism that has never been cultured, Tropheryma whipplei, was identified using this approach.


DNA sample
Denatured ssDNA
gene of interest
DNA probes
molecular beacon
1 Isolate DNA from body fluid sample.
2 Denature DNA sample and combine with DNA probes. Probes are complementary to the gene of interest and labeled with a molecular beacon.
3 DNA probes will bind to the gene of interest if it is present in the DNA sample.

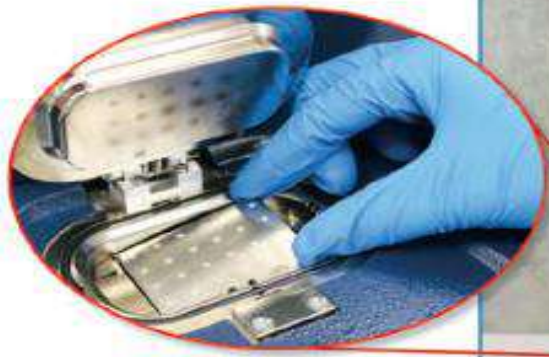
Proteomic tests (MALDI-TOF-MS)

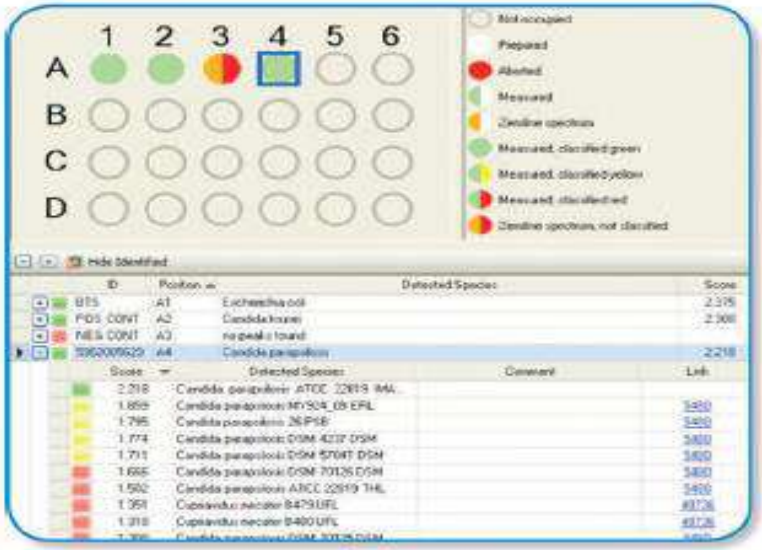
- MALDI-TOF (Matrix Assisted Laser Desorption/Ionization Time of Flight) is an automated mass spectrometry and software system designed for :
- Rapid microbial identification, Detection of Antibiotic Resistance in Bacteria
- Viral Identification, Genotyping, Subtyping, and epidemiological Studies
- Identification of Fungi (mold and yeast) and detection of Antibiotic Resistance in Fungi





Add Formic Acid and Matrix and Dry





Mayo Clinic
Laboratory Services Report

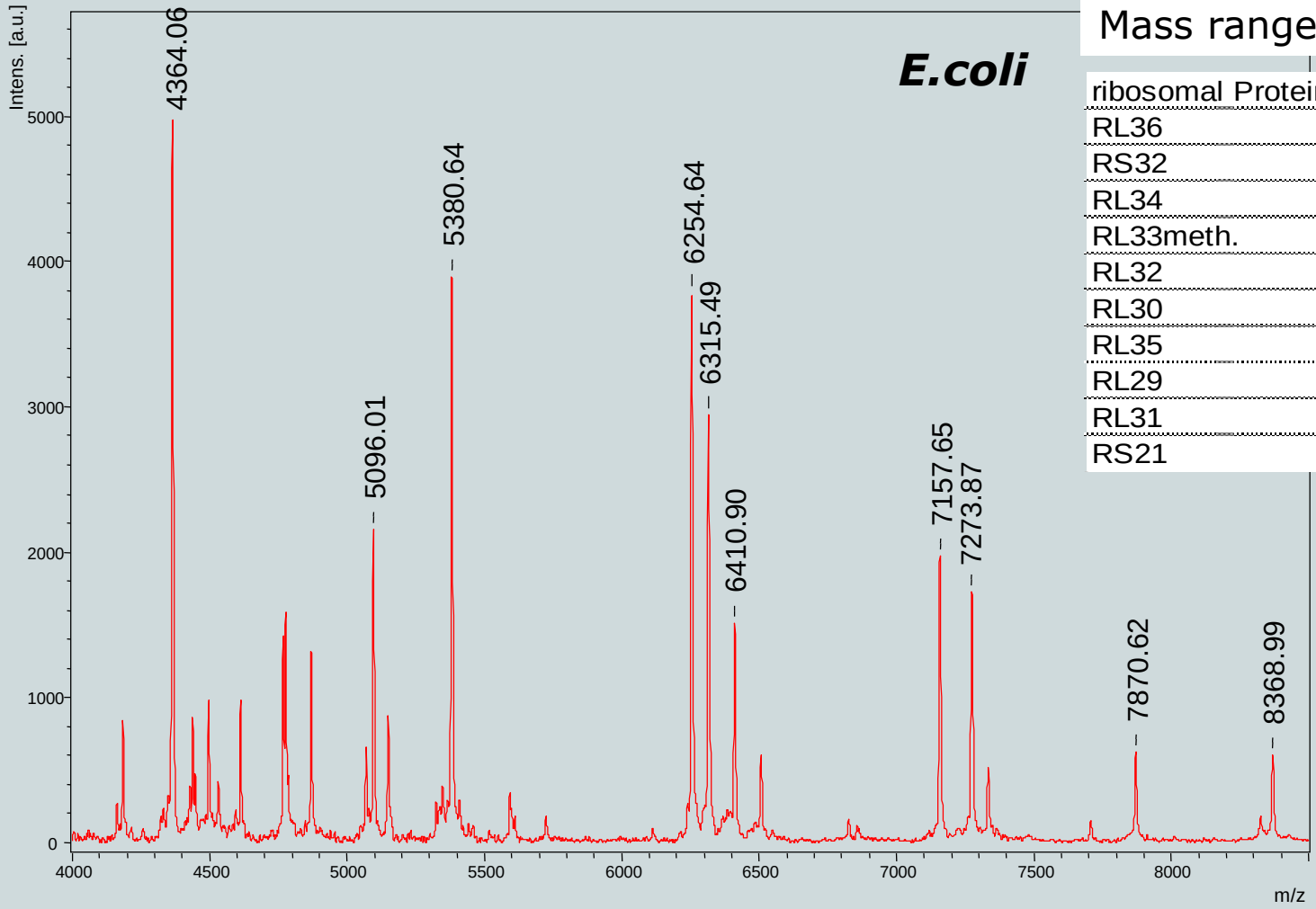
MICROBIOLOGY

Fungal Culture, Routine FINAL 07/25/2012 14:42 MCR

CANDIDA PARAPSILOSIS Many
Identified by mass spectrometry

MCR Mayo Clinic Dept.of Laboratory Medicine and Pathology, 200 First Street SW,Rochester,MN 55905 Franklin R.Cockerill, M.D., Lab Director

MALDI-TOF MS profile spectrum



E.coli

Positive linear mode
Mass range 2-20 kDa

ribosomal Protein	m/z
RL36	4364,33
RS32	5095,82
RL34	5380,39
RL33meth.	6255,39
RL32	6315,19
RL30	6410,60
RL35	7157,74
RL29	7273,45
RL31	7871,06
RS21	8368,76

Several important steps precede the actual laboratory work,

- 1) choosing the appropriate specimen to examine, which requires an understanding of the pathogenesis of the infection;
- (2) obtaining the specimen properly to avoid contamination from the normal flora;
- (3) transporting the specimen promptly to the laboratory or storing it correctly;
- (4) providing essential information to guide the laboratory personnel.

Three approaches to the bacteriologic laboratory work:

- (1) Observing the organism in the microscope after staining.
- (2) Obtaining a pure culture of the organism by inoculating
- it onto a bacteriologic medium.
- (3) Identifying the organism by using biochemical reactions, growth on selective media, DNA probes, or specific

TABLE 9–1 General Approach to the Diagnosis of a Bacterial Infection

1. Obtain a specimen from the infected site.
2. Stain the specimen using the appropriate procedure (e.g., Gram stain or acid-fast stain). If bacteria are seen in the Gram stain specimen, their shape (e.g., cocci or rods), size, arrangement (e.g., chains or clusters), and whether they are gram-positive or gram-negative should be observed. It is also important to determine whether only one or more than one type of bacteria is present. The microscopic appearance is *not* sufficient to speciate an organism, but it often allows an educated guess to be made regarding the genus of the organism and thereby guides empiric therapy.
3. Culture the specimen on the appropriate media (e.g., blood agar plates). In most instances, the plates should be streaked in such a manner to obtain isolated colonies (i.e., a "pure culture"). The plates should be incubated in the presence or absence of oxygen as appropriate.
4. Identify the organism using the appropriate tests (e.g., sugar fermentation, DNA probes, antibody-based tests such as agglutination, or immunofluorescence). Note special features such as hemolysis and pigment formation.
5. Perform antibiotic susceptibility tests.

TABLE 9–2 How to Diagnose a Bacterial Infection When the Culture Is Negative

1. Detect antibody in the patient's serum. Detection of immunoglobulin (Ig) M antibody indicates a current infection. A fourfold or greater rise in antibody titer between the acute serum sample and the convalescent serum sample also indicates a current infection. (A major drawback with the use of acute and convalescent serum samples is that the convalescent sample is usually taken 10–14 days after the acute sample. By this time, the patient has often recovered and the diagnosis becomes a retrospective one.) A single IgG antibody titer is difficult to interpret because it is unclear whether it represents a current or a previous infection. In certain diseases, a single titer of sufficient magnitude can be used as presumptive evidence of a current infection.
2. Detect antigen in the patient's specimen. Use known antibody to detect presence of antigens of the organisms (e.g., fluorescent antibody to detect antigens in tissue, latex agglutination to detect capsular polysaccharide antigens in spinal fluid).
3. Detect nucleic acids in the patient's specimen. Use polymerase chain reaction (PCR) and DNA probes to detect the DNA or RNA of the organism.

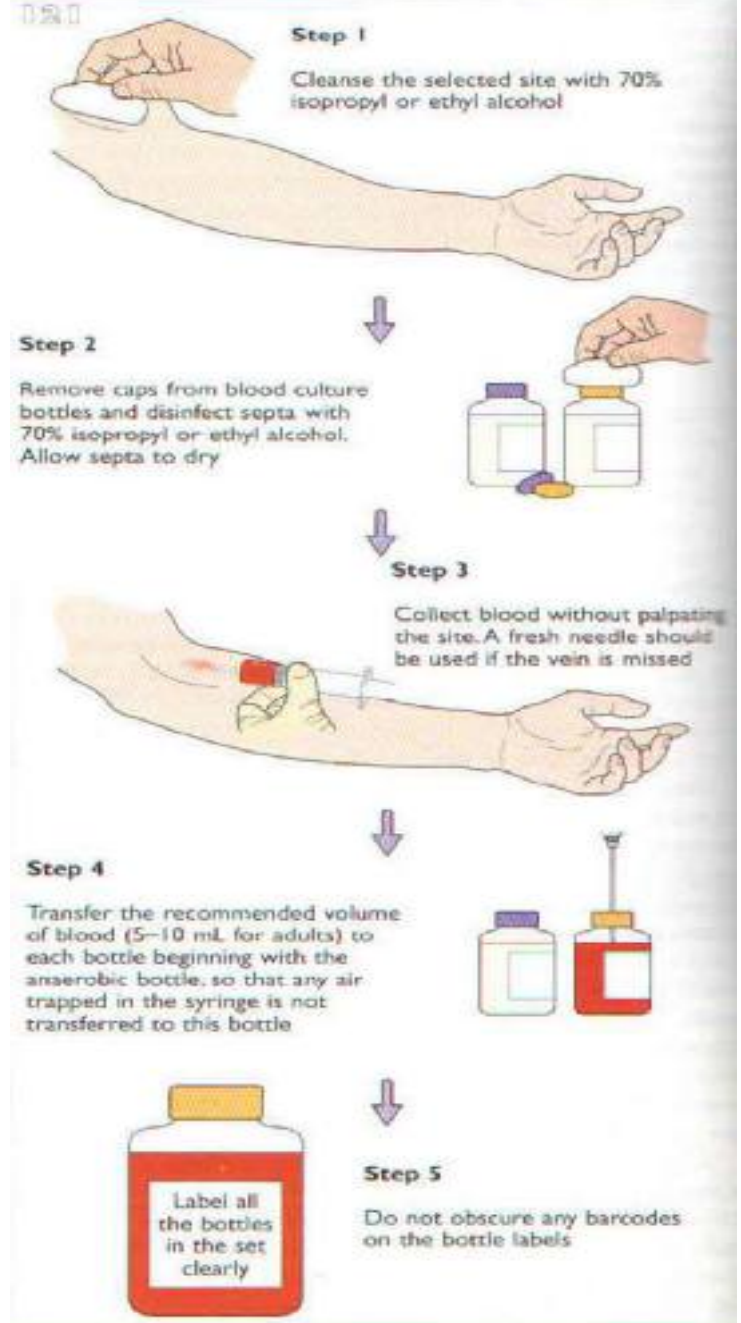
Approach to laboratory diagnosis

- Blood agar is used because it supports the growth of many bacteria and the type of hemolysis can be observed.
- Blood agar contains red blood cells, but it should be noted that viruses and obligate intracellular bacteria, such as Chlamydia and Rickettsia, will not grow on blood agar
- Red blood cells do not have a functioning nucleus and, therefore, are incapable of supporting the growth of either viruses or the obligate intracellular bacteria.
- Blood agar contains inhibitors for certain bacteria, such as members of the Neisseria and Haemophilus genera, and the blood must be heated to inactivate these inhibitors.

- These bacteria, therefore, are grown on cooked blood agar or chocolate agar (so named because the heated blood turns a chocolate color).
- Certain other media, called "selective, differential" media, are often used.
- These media are selective because they contain compounds that selectively allow certain bacteria to grow and
- differential because they contain other compounds that allow one type of bacteria to be differentiated from another based on some biochemical reaction.

Blood Cultures

- Blood cultures are performed most often when sepsis, endocarditis, osteomyelitis, meningitis, or pneumonia is suspected.
- The organisms most frequently isolated from blood cultures are two gram-positive cocci,
- Staphylococcus aureus and
- Streptococcus pneumoniae, and
- three gram negative rods,
- Escherichia coli,
- Klebsiella pneumoniae,
- and Pseudomonas aeruginosa.



121 The collection of blood cultures requires good aseptic technique in order to reduce the chance of contamination.

- It is important to obtain at least three 10-mL blood samples in a 24-hour period because the number of organisms can be small and their presence intermittent.
- The site for venipuncture must be cleansed with 2% iodine to prevent contamination by members of the flora of the skin,
- usually Staphylococcus epidermidis.
- The blood obtained is added to 100 mL of a rich growth medium such as brain– heart infusion broth.
- Whether one or two bottles are inoculated varies among hospitals.
- If two bottles are used, one is kept under anaerobic conditions and the other is not.
- If one bottle is used, the low oxygen tension at the bottom of the bottle permits anaerobes to grow.

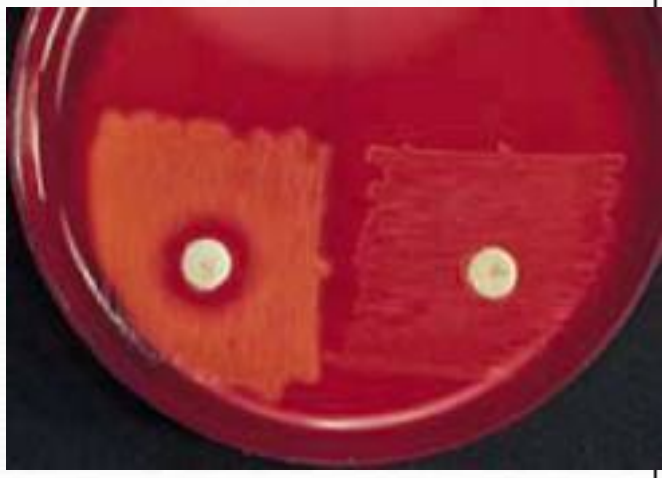
- Blood cultures are checked for turbidity or for CO_2 production daily for 7 days or longer.
- If growth occurs, Gram stain, subculture, and antibiotic sensitivity tests are performed.
- If no growth is observed after 1 or 2 days, blind subculturing onto other media may reveal organisms.
- Cultures should be held for 14 days when infective endocarditis, fungemia, or infection by slow-growing bacteria (e.g., Brucella) is suspected.


BD BACTEC FX40

Throat Cultures

- Throat cultures are used primarily to detect the presence of group A β-hemolytic streptococci (Streptococcus pyogenes), an important and treatable cause of pharyngitis.
- They are also used when diphtheria, gonococcal pharyngitis, or thrush (Candida) is suspected.
- When the specimen is being obtained, the swab should touch not only the posterior pharynx, but also both tonsils or tonsillar fossae as well.

- The material on the swab is inoculated onto a blood agar plate and streaked to obtain single colonies.
- If colonies of β-hemolytic streptococci are found after 24 hours of incubation at 35°C, a bacitracin disk is used to determine whether the organism is likely to be a group A streptococcus.
- If growth is inhibited around the disk, it is a group A streptococcus; if not, it is a non–group A β-hemolytic streptococcus.
- Gram stain is typically not done on a throat swab because it is impossible to distinguish between the appearance of the normal flora streptococci and S. pyogenes



Group A streptococci is susceptible to Bacitracin disk (left); The right shows resistance

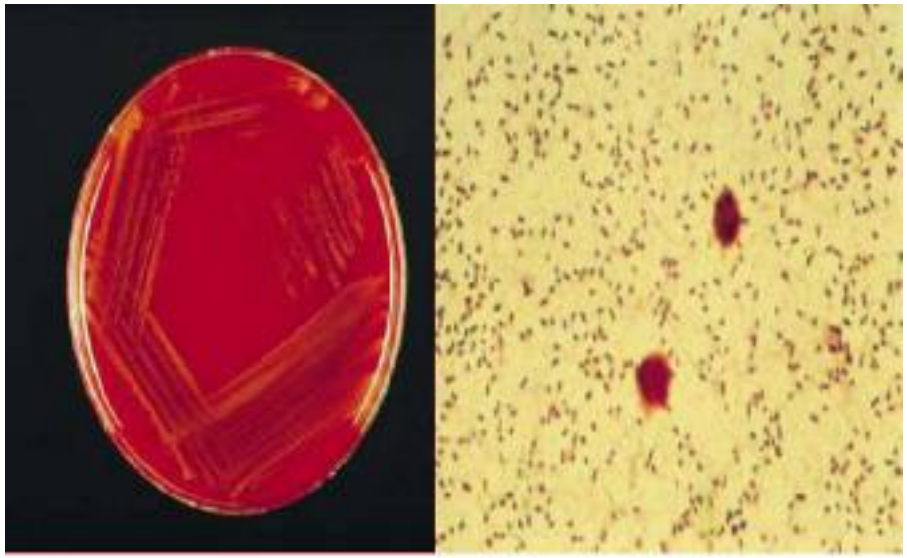
Sputum Cultures

- Sputum cultures are performed primarily when pneumonia or tuberculosis is suspected.
- The most frequent cause of community-acquired pneumonia is S. pneumoniae, whereas S. aureus and gram-negative rods, such as K. pneumonia and P. aeruginosa, are common causes of hospital-acquired pneumonias.
- It is important that the specimen for culture really be sputum, not saliva.
- Examination of a gram-stained smear of the specimen frequently reveals whether the specimen is satisfactory.
- A reliable specimen has more than 25 leukocytes and fewer than 10 epithelial cells per 100× field.

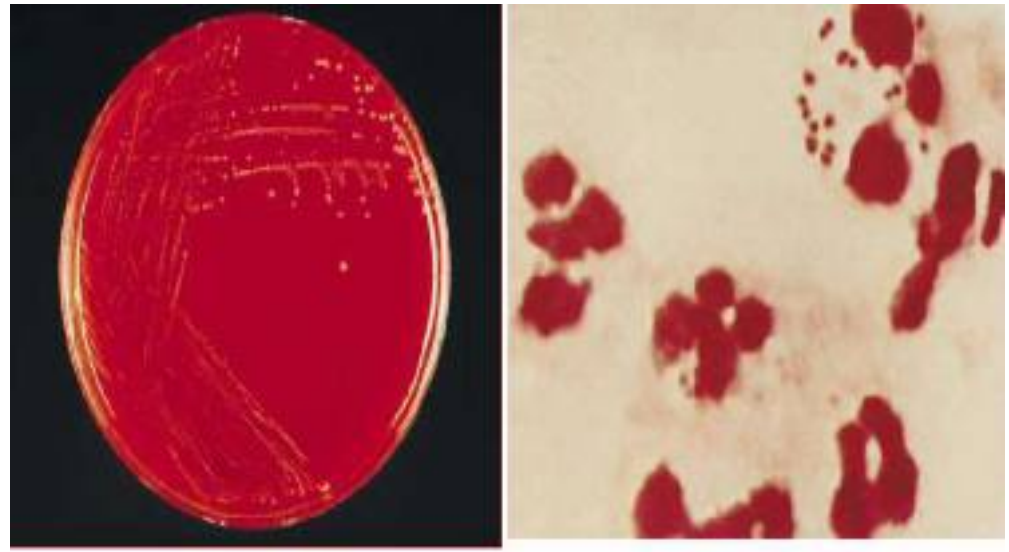
- the patient cannot cough and the need for a microbiologic diagnosis is strong, induction of sputum, transtracheal aspirate, bronchial lavage, or lung biopsy may be necessary.
- Because these procedures bypass the normal flora of the upper airway, they are more likely to provide an accurate microbiologic diagnosis
- Cultures of Mycoplasma are infrequently done; diagnosis is usually confirmed by a rise in antibody titer.
- If Legionella pneumonia is suspected, the organism can be cultured on charcoal-yeast agar, which contains the high concentrations of iron and sulfur required for growth.
- If tuberculosis is suspected, an acid-fast stain should be done immediately and the sputum cultured on special media, which are incubated for at least 6 weeks.

Spinal Fluid Cultures

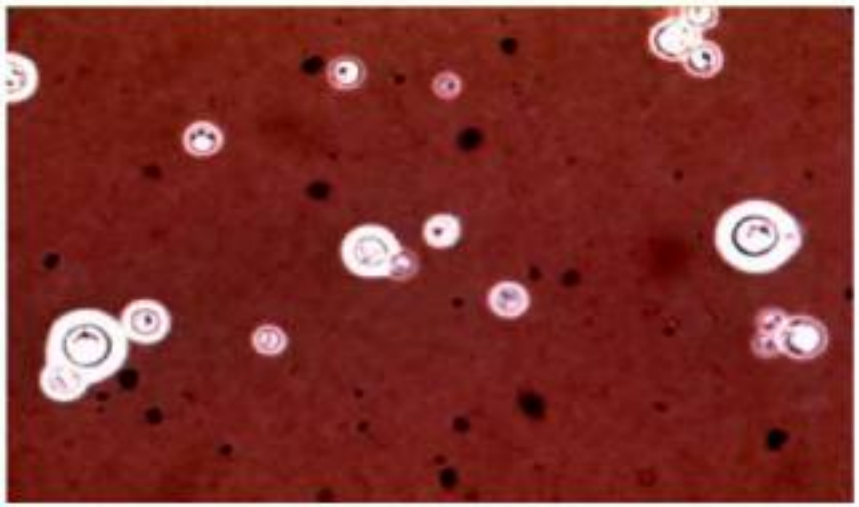
- Spinal fluid cultures are performed primarily when meningitis is suspected.
- Spinal fluid specimens from cases of encephalitis, brain abscess, and subdural empyema usually show negative cultures.
- The most important causes of acute bacterial meningitis are three encapsulated organisms: Neisseria meningitidis, S. pneumoniae, and Haemophilus influenzae.
- Because acute meningitis is a medical emergency, the specimen should be taken immediately to the laboratory.
- The gram-stained smear of the sediment of the centrifuged sample guides the immediate empirical treatment.



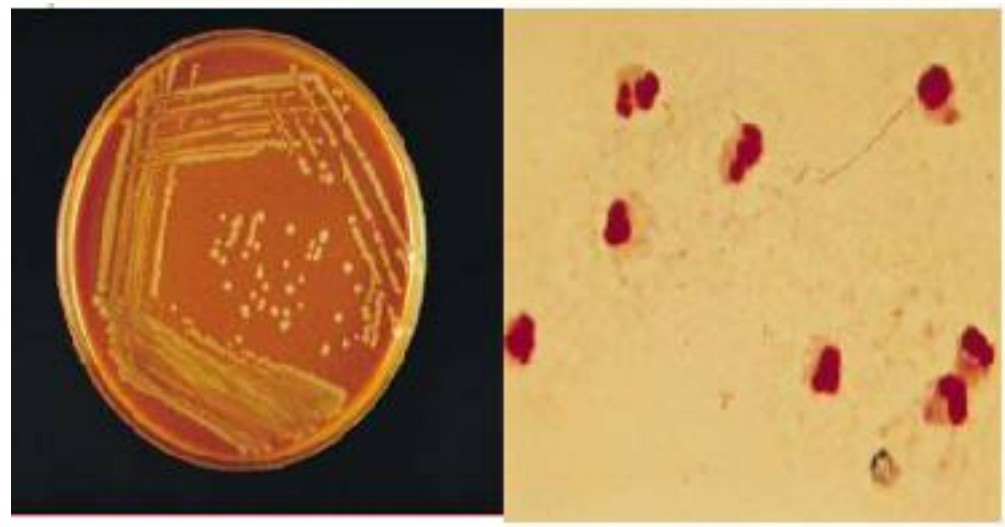
S. pneumoniae



N. meningitidis



Cryptococcus neoformans. India ink staining of CSF



H. influenzae

- If organisms resembling N. meningitidis, H. influenzae, or S. pneumoniae are seen, the quelling test or immunofluorescence with specific antisera can identify the organism rapidly.
- Cultures are done on blood and on chocolate agar and incubated at 35°C in a 5% CO2 atmosphere.
- Hematin and nicotinamide adenine dinucleotide (NAD) (factors X and V, respectively) are added to enhance the growth of H. influenzae.
- In cases of subacute meningitis, Mycobacterium tuberculosis and the fungus Cryptococcus neoformans are the most common organisms isolated.
- Acid-fast stains of the spinal fluid should be performed, The fluid should be cultured and the cultures held for a minimum of 6 weeks.

- Crytococcus neoformans, a budding yeast with a prominent capsule, can be seen in spinal fluid when India ink is used.
- Immunologic tests to detect the presence of capsular antigen in the spinal fluid can be used to identify N. meningitidis, S. pneumoniae, H. influenzae, group B streptococci, E. coli, and C. neoformans.
- The two tests most frequently used are latex particle agglutination and counterimmunoelectrophoresis.

Stool Cultures

- Stool cultures are performed primarily for cases of enterocolitis.
- The most common bacterial pathogens causing diarrhea in the United States are Shigella, Salmonella, and Campylobacter.
- E. coli O157 strains are also an important cause of diarrhea.
- A direct microscopic examination of the stool can be informative from two points of view:

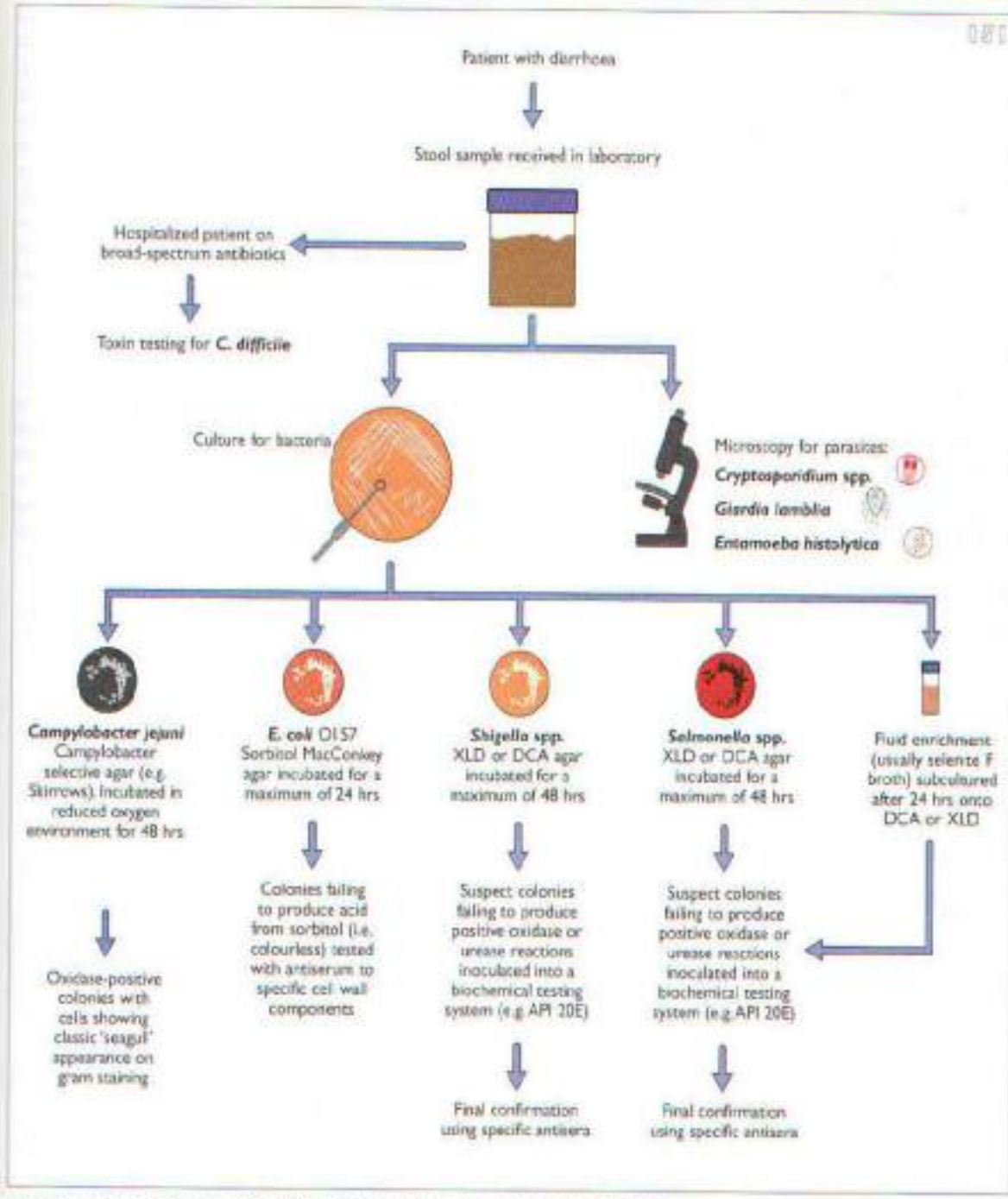
(1) a methylene blue stain that reveals many leukocytes indicates that an invasive organism rather than a toxigenic one is involved,

(2) A Gram stain may reveal large numbers of certain organisms, such as staphylococci, clostridia, or campylobacters.

- Gram stain of the stool is not usually done because the large numbers of bacteria in the normal flora of the colon make the interpretation difficult.

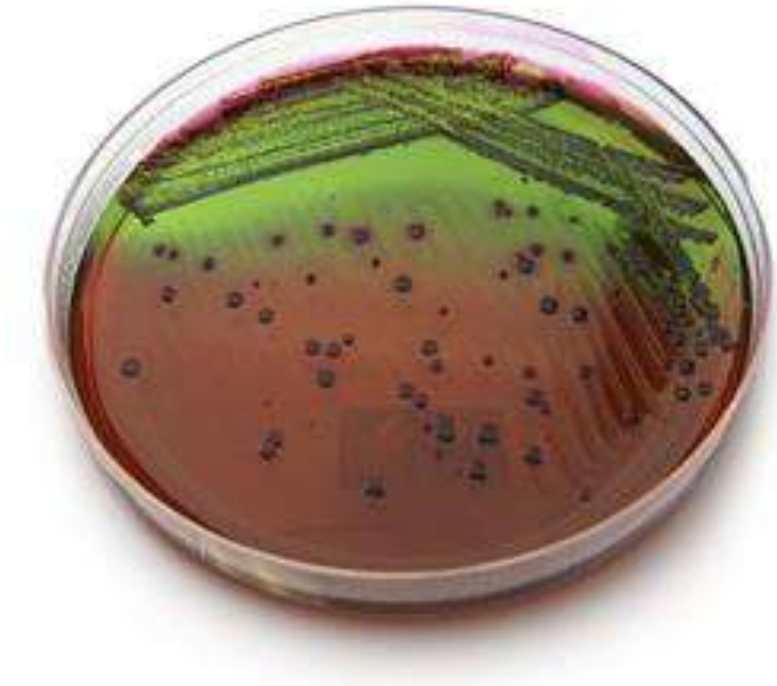
- For culture of Salmonella and Shigella, a selective, differential medium such as MacConkey or Eosin–Methylene Blue (EMB) agar is used
- On EMB agar, colonies of E. coli, a lactose fermenter, appear purple and have a green sheen.
- In contrast, colonies of non–lactose fermenters, such as Salmonella and Shigella, appear colorless.
- Some species of Proteus resemble Salmonella on TSI agar but can be distinguished because they produce the enzyme urease, whereas Salmonella does not.
- The organism is further identified as either a Salmonella or a Shigella species by using a specific antisera to the organism's cell wall O antigen in an agglutination test.

- Campylobacter jejuni is cultured on antibiotic-containing media (e.g., Skirrow's agar) at 42°C in an atmosphere containing 5% O2 and 10% CO2.
- Stool cultures are infrequently performed for organisms such as Yersinia enterocolitica, Vibrio parahaemolyticus, and enteropathic or toxigenic E. coli.
- Stool specimens that are grossly bloody are typically cultured on MacConkey-sorbitol media.
- E. coli O157 strains do not ferment sorbitol and appear as colorless colonies, whereas typical E. coli strains do ferment sorbitol and appear red.



151 When a stool specimen is submitted to the laboratory, various procedures are conducted.

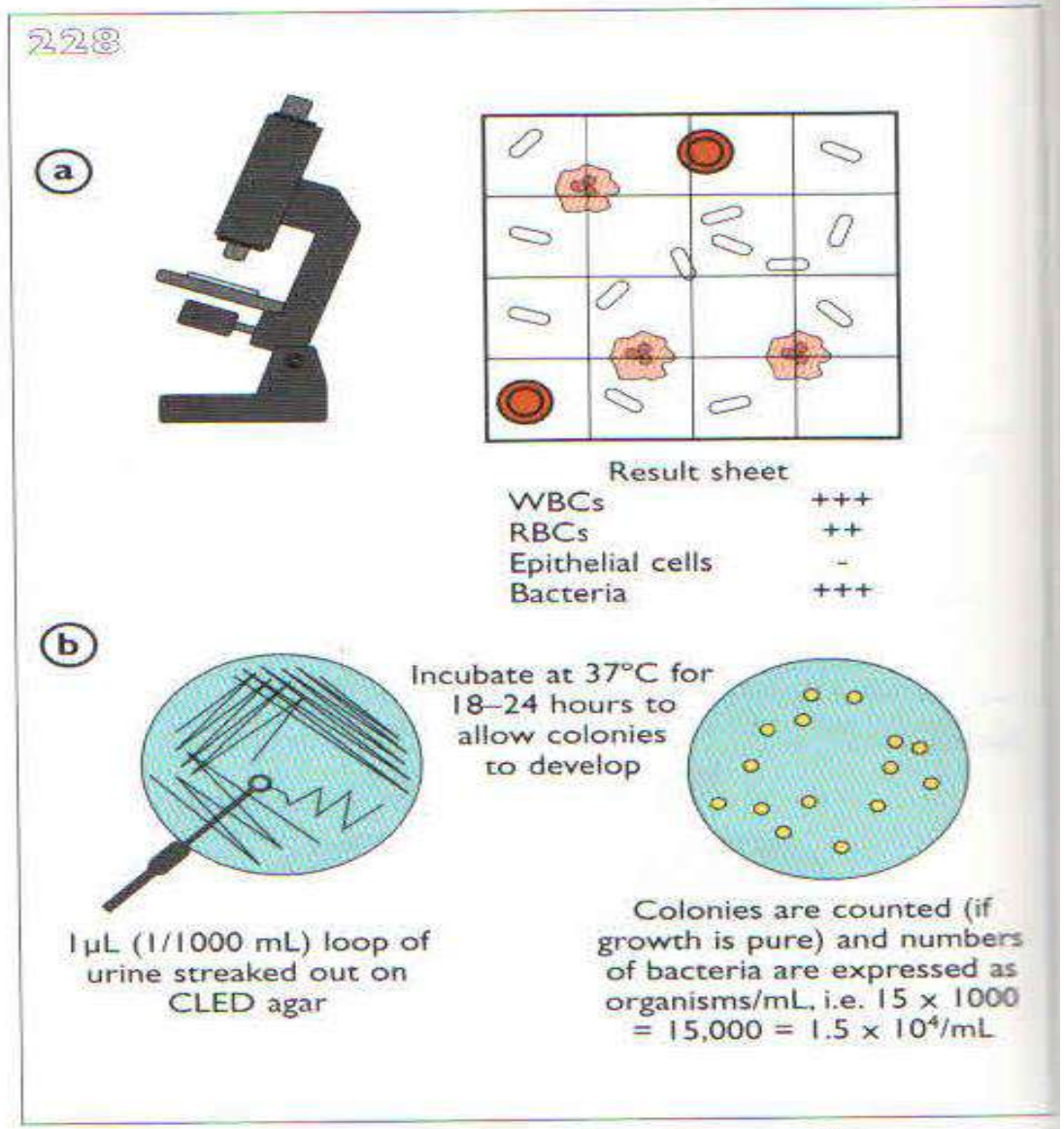
Sorbitol MacConkey Agar: Sorbitol replaces lactose in MacConkey. E. coli O157:H7 does not ferment sorbitol, whereas other species of E. coil are positive for fermentation of sorbitol. This makes the media a good screen for O157:H7.



Eosin–Methylene Blue (EMB) agar

Urine Cultures

- Urine cultures are performed primarily when pyelonephritis or cystitis is suspected.
- The most frequent cause of urinary tract infections is E. coli. Other common agents are Enterobacter, Proteus, and Enterococcus faecalis.
- Urine in the bladder of a healthy person is sterile, but it acquires organisms of the normal flora as it passes through the distal portion of the urethra.
- To avoid these organisms, a midstream specimen, voided after washing the external orifice, is used for urine cultures.
- In special situations, suprapubic aspiration or catheterization may be required to obtain a specimen.



228 (**a**) Urine microscopy can be used to determine the presence of white blood cells (WBCs) and red blood cells (RBCs), epithelial cells, and bacteria. (**b**) A volume of urine is plated out onto a selective medium such as CLED.

- Because urine is a good culture medium, it is essential that the cultures be done within 1 hour after collection or stored in a refrigerator at 4°C for no more than 18 hours.
- It is commonly accepted that a bacterial count of at least 100,000/mL must be found to conclude that significant bacteriuria is present (in asymptomatic persons).
- There is evidence that as few as 100/mL are significant in symptomatic patients.
- For this determination to be made, quantitative or semiquantitative cultures must be performed

There are several techniques:

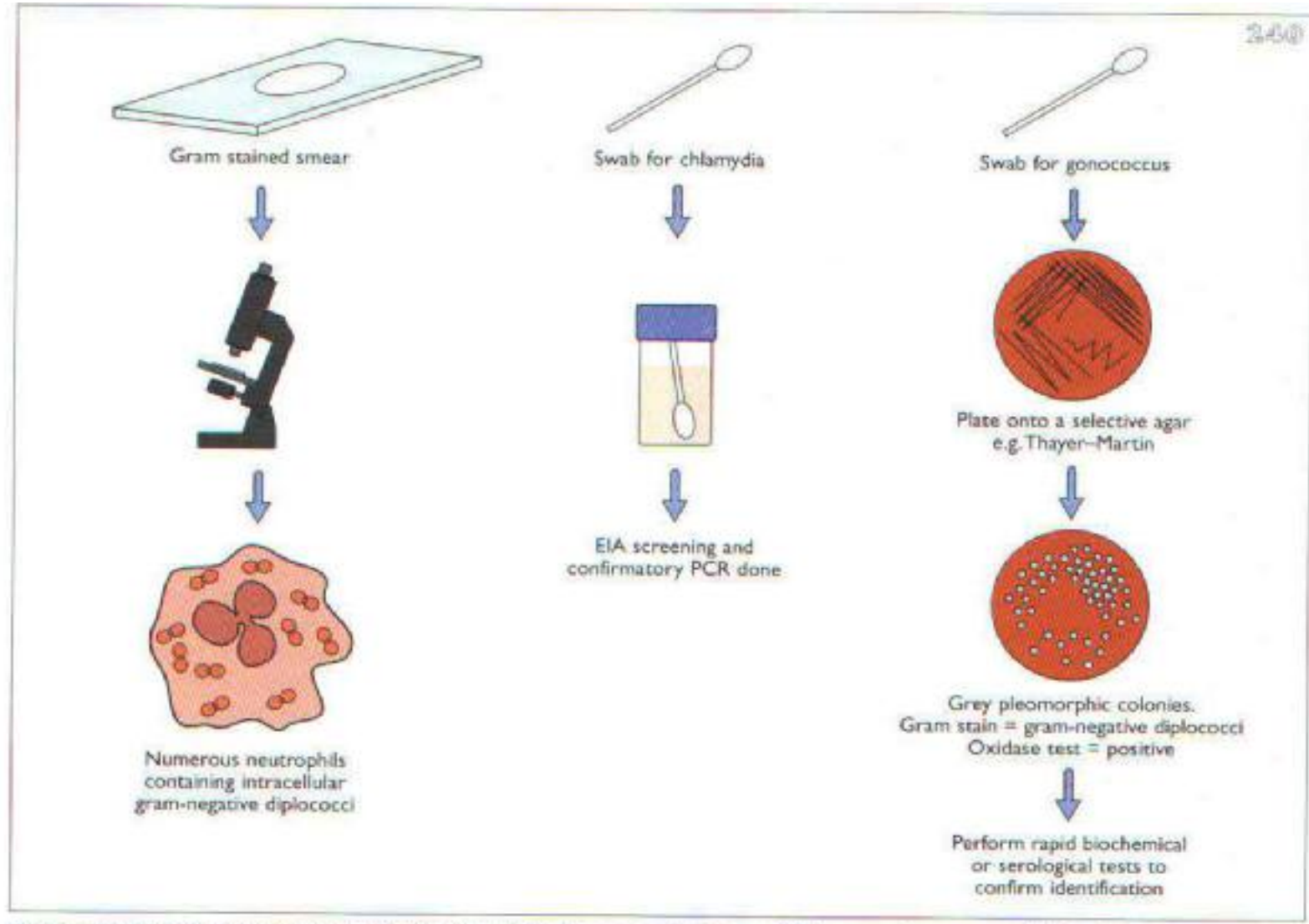
- (1) a calibrated loop that holds 0.001 mL of urine can be used to streak the culture;
- (2) serial 10-fold dilutions can be made and samples from the dilutions streaked; and
- (3) a screening procedure suitable for the physician's office involves an agar-covered "paddle" that is dipped into the urine—after the paddle is incubated, the density of the colonies is compared with standard charts to obtain an estimate of the concentration of bacteria.

Genital Tract Cultures

- Genital tract cultures are performed primarily on specimens from individuals with an abnormal discharge or on specimens from asymptomatic contacts of a person with a sexually transmitted disease.
- One of the most important pathogens in the genital tract is Neisseria gonorrhoeae.
- The laboratory diagnosis of gonorrhea is made by microscopic examination of a gram-stained smear and by culture of the organism.

- Specimens are obtained by swabbing the urethral canal (for men), the cervix (for women), or the anal canal (for men and women).
- A urethral discharge from the penis is frequently used.
- Because N. gonorrhoeae is very delicate, the specimen should be inoculated directly onto a Thayer-Martin chocolate agar plate or onto a special transport medium (e.g., Trans-grow).
- Gram-negative diplococci found intracellularly within neutrophils on a smear of a urethral discharge from a man have over 90% probability of being N. gonorrhoeae.
- Because smears are less reliable when made from swabs of the endocervix and anal canal, cultures are necessary.

- The finding of only extracellular diplococci suggests that these Neisseriae may be members of the normal flora and that the patient may have nongonococcal urethritis
- Nongonococcal urethritis and cervicitis are also extremely common infections.
- The most frequent cause is Chlamydia trachomatis, which cannot grow on artificial medium but must be grown in living cells.
- For this purpose, cultures of human cells or the yolk sacs of embryonated eggs are used
- The finding of typical intracytoplasmic inclusions when using Giemsa stain or fluorescent antibody is diagnostic.



240 An outline of the methods used to identify chlamydial and gonococcal infections. (EIA: enzyme immunoassay; PCR: polymerase chain reaction.)

- Because of the difficulty of culturing C. trachomatis, nonbacteriologic methods, such as enzyme linked immunosorbent assay (ELISA) to detect chlamydial antigens in exudates or urine or
- DNA probe assays to detect chlamydial nucleic acids (nucleic acid amplification test, NAAT), are now often used to diagnose sexually transmitted diseases caused by this organism.
- Because Treponema pallidum, the agent of syphilis, cannot be cultured, diagnosis is made by microscopy and serology.
- The presence of motile spirochetes with typical morphologic features seen by darkfield microscopy of the fluid from a painless genital lesion is sufficient for the diagnosis

The serologic tests fall into two groups:

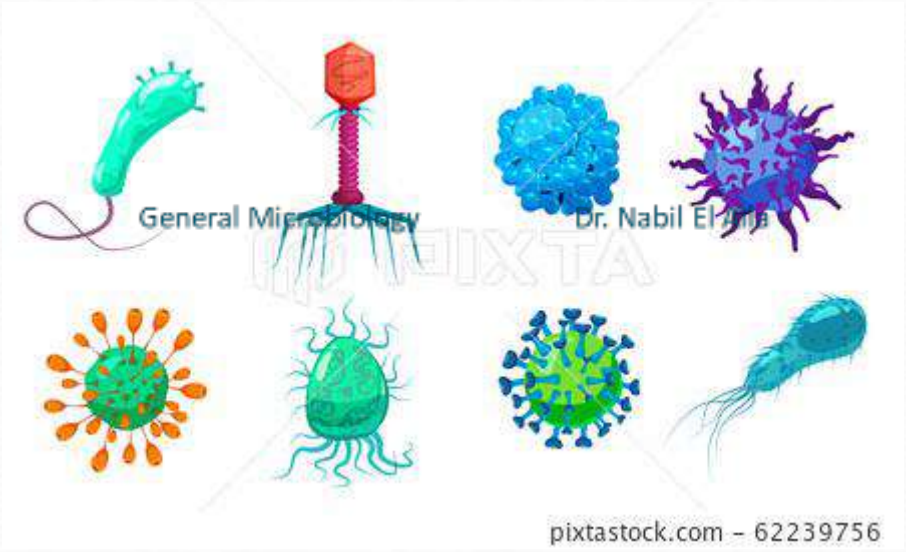
- (1) the nontreponemal antibody tests such as the Venereal Disease Research Laboratory (VDRL) or rapid plasma reagin (RPR) test and
- (2) the treponemal antibody tests such as the fluorescent
- treponemal antibody-absorption (FTA-ABS) test.

Wound & Abscess Cultures

- A great variety of organisms are involved in wound and abscess infections.
- The bacteria most frequently isolated differ according to anatomic site and predisposing factors.
- Abscesses of the brain, lungs, and abdomen are frequently caused by anaerobes such as Bacteroides fragilis and gram Positive cocci such as S. aureus and S. pyogenes.
- Traumatic open-wound infections are caused primarily by members of the soil flora such as Clostridium perfringens; surgical wound infections are usually due to S. aureus.

- Infections of dog or cat bites are commonly due to Pasteurella multocida, whereas human bites primarily involve the mouth anaerobes.
- Because anaerobes are frequently involved in these types of infections, it is important to place the specimen in anaerobic collection tubes and transport it promptly to the laboratory
- Because many of these infections are due to multiple organisms, including mixtures of anaerobes and non anaerobes, it is important to culture the specimen on several different media under different atmospheric conditions.

End of Lecture



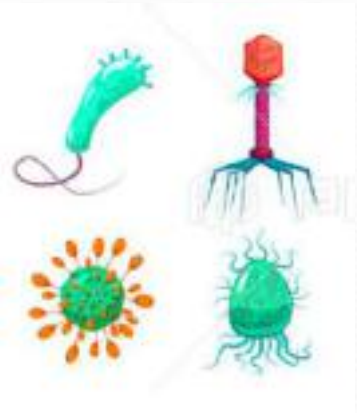
General Microbiology

Antimicrobial drugs
Mechanism of Action

Dr. Nabil A. ElAila

Associate Professor of Molecular Microbiology

Al Aqsa University - Gaza , Palestine



Antimicrobial stewardship

- The worldwide problem of antibiotic resistance makes the need for antimicrobial stewardship evident.
- These pathogens include methicillin-resistant Staphylococcus aureus and extended-spectrum β-lactamase producing gram-negative rods (e.g., Escherichia coli and Klebsiella pneumoniae).
- Hospital–associated infections, many of which are caused by antibiotic-resistant bacteria, are estimated to cost billions of dollars each year.

The basic principles of good stewardship are threefold:

- (1) Reduce inappropriate use of antibiotics,
- (2) Encourage targeted treatment with narrow spectrum drugs,
- (3) limit adverse effects

Inappropriate antibiotic use can occur for many reasons,

- Inappropriate antibiotic use can occur for many reasons, including the
- providers' desire to adhere to patient's wishes, even when it is not medically appropriate.
- The most common example of inappropriate antibiotic use is the prescribing of antibiotics for a viral respiratory tract infection.

Principles of antimicrobial therapy

- The most important concept underlying antimicrobial therapy is selective toxicity (i.e., selective inhibition of the growth of the microorganism without damage to the host).
- Selective toxicity is achieved by exploiting the differences between the metabolism and structure of the microorganism and the corresponding features of human cells.
- For example, penicillins and cephalosporins are effective antibacterial agents because they prevent the synthesis of peptidoglycan, thereby inhibiting the growth of bacterial but not human cells.

- **Broad-spectrum** antibiotics are active against several
- types of microorganisms (e.g., tetracyclines are active
- against many gram-negative rods, chlamydiae, mycoplasmas,
- and rickettsiae).
- **Narrow-spectrum** antibiotics are active against one or very few types (e.g., vancomycin is primarily used against certain gram-positive cocci, namely, staphylococci and enterococci).

Bactericidal & bacteriostatic activity

- A bactericidal drug kills bacteria, whereas a bacteriostatic drug inhibits their growth but does not kill them.
- The salient features of the behavior of bacteriostatic drugs are
- That: (1) the bacteria can grow again when the drug is withdrawn,
- and (2) host defense mechanisms, such as phagocytosis, are required to kill the bacteria.
- Bactericidal drugs are particularly useful in certain infections (e.g., those that are immediately life-threatening; those in patients whose polymorphonuclear leukocyte count is below 500/µL; and endocarditis, in which phagocytosis is limited by the fibrinous network of the vegetation and bacteriostatic drugs do not affect a cure).

TABLE 10–2 Mechanism of Action of Important Antibacterial and Antifungal Drugs

Mechanism of Action	Drugs
Inhibition of cell wall synthesis	
1. (a) Antibacterial activity inhibition of cross-linking (transpeptidation) of peptidoglycan	Penicillins, cephalosporins, imipenem, aztreonam, vancomycin
(b) Inhibition of other steps in peptidoglycan synthesis	Cycloserine, bacitracin
2. Antifungal activity inhibition of β-glucan synthesis	Caspofungin
Inhibition of protein synthesis	
Action on 50S ribosomal subunit	Chloramphenicol, erythromycin, clindamycin, linezolid
Action on 30S ribosomal subunit	Tetracyclines and aminoglycosides
Inhibition of nucleic acid synthesis	
Inhibition of nucleotide synthesis	Sulfonamides, trimethoprim
Inhibition of DNA synthesis	Quinolones (e.g., ciprofloxacin)
Inhibition of mRNA synthesis	Rifampin
Alteration of cell membrane function	
Antibacterial activity	Polymyxin, daptomycin
Antifungal activity	Amphotericin B, nystatin, terbinafine, azoles (e.g., itraconazole)
Other mechanisms of action	
1. Antibacterial activity	Isoniazid, metronidazole, ethambutol, pyrazinamide
2. Antifungal activity	Griseofulvin, pentamidine

MECHANISMS OF ACTION

- 1. Inhibition of Bacterial Cell Wall Synthesis

Penicillins

- Penicillins (and cephalosporins) act by inhibiting transpeptidases,
- the enzymes that catalyze the final cross-linking step in the synthesis of peptidoglycan.

For example, in S. aureus, transpeptidation occurs between the amino group on the end of the pentaglycine cross-link and the terminal carboxyl group of the d-alanine on the tetrapeptide side chain.

- Because the stereochemistry of penicillin is similar to that of a dipeptide, d-alanyl-d-alanine, penicillin can bind to the active site of the transpeptidase and inhibit its activity.

Two additional factors are involved in the action of penicillin:

- (1) The first is that penicillin binds to a variety of receptors in the bacterial cell membrane and cell wall, called penicillin-binding proteins (PBPs).
- Some PBPs are transpeptidases; others function in the synthesis of peptidoglycan.
- Changes in PBPs are in part responsible for an organism's becoming resistant to penicillin.
- (2) The second factor is that autolytic enzymes called murein hydrolases (murein is a synonym for peptidoglycan) are activated in penicillin-treated cells and degrade the peptidoglycan.

- Some bacteria (e.g., strains of S. aureus) are tolerant to the action of penicillin, because these autolytic enzymes are not activated.
- A tolerant organism is one that is inhibited but not killed by a drug that is usually bactericidal, such as penicillin

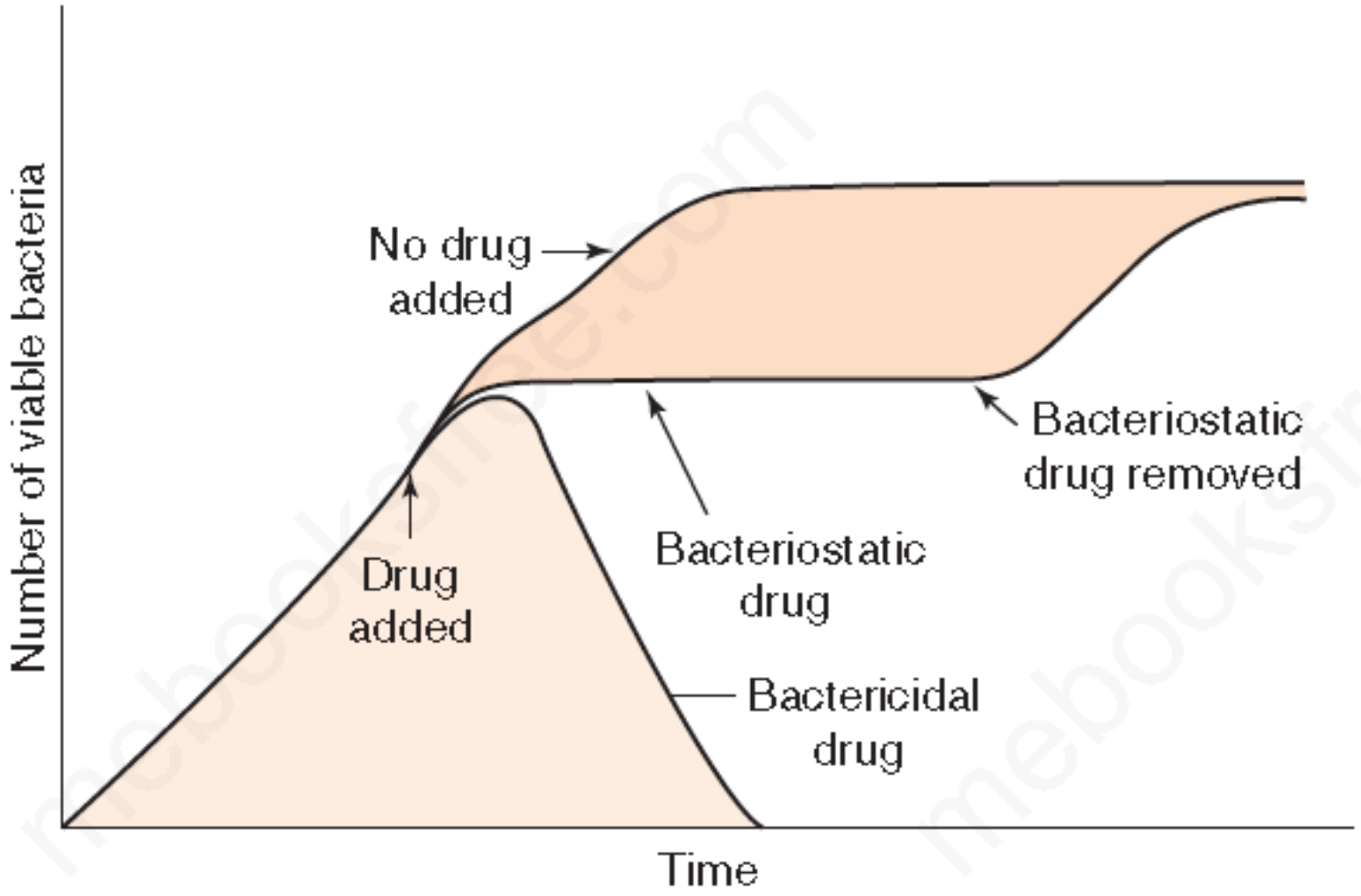


FIGURE 10–1 Bactericidal and bacteriostatic activity of antimicrobial drugs. Either a bactericidal or a bacteriostatic drug is added

- Penicillin is bactericidal, but it kills cells only when they are growing. When cells are growing, new peptidoglycan is being synthesized, and transpeptidation occurs.
- Penicillins are therefore more active during the log phase of bacterial cell growth than during the stationary phase
- Penicillins (and cephalosporins) are called β-lactam drugs because of the importance of the β-lactam ring
- An intact ring structure is essential for antibacterial activity; cleavage of the ring by penicillinases (β-lactamases) inactivates the drug.
- The most important naturally occurring compound is benzylpenicillin (penicillin G),

- **Penicillin G is available in three main forms:**
- (1) Aqueous penicillin G, which is metabolized most rapidly.
- (2) Procaine penicillin G, in which penicillin G is conjugated to procaine. This form is metabolized more slowly and is less painful when injected intramuscularly because the procaine acts as an anesthetic.
- (3) Benzathine penicillin G, in which penicillin G is conjugated to benzathine. This form is metabolized very slowly and is often called a "depot" preparation.

- Benzylpenicillin is one of the most widely used and effective antibiotics. However, it has four disadvantages, three of which have been successfully overcome by chemical modification of the side chain.
- 1) limited effectiveness against many gram-negative rods,
- (2) hydrolysis by gastric acids, so that it cannot be taken orally,
- (3) inactivation by β-lactamases.
- The limited effectiveness of penicillin G against gram-negative rods is due to the inability of the drug to penetrate the outer membrane of the organism.
- The fourth disadvantage common to all penicillins that has not been overcome is hypersensitivity, especially anaphylaxis, in some recipients of the drug.

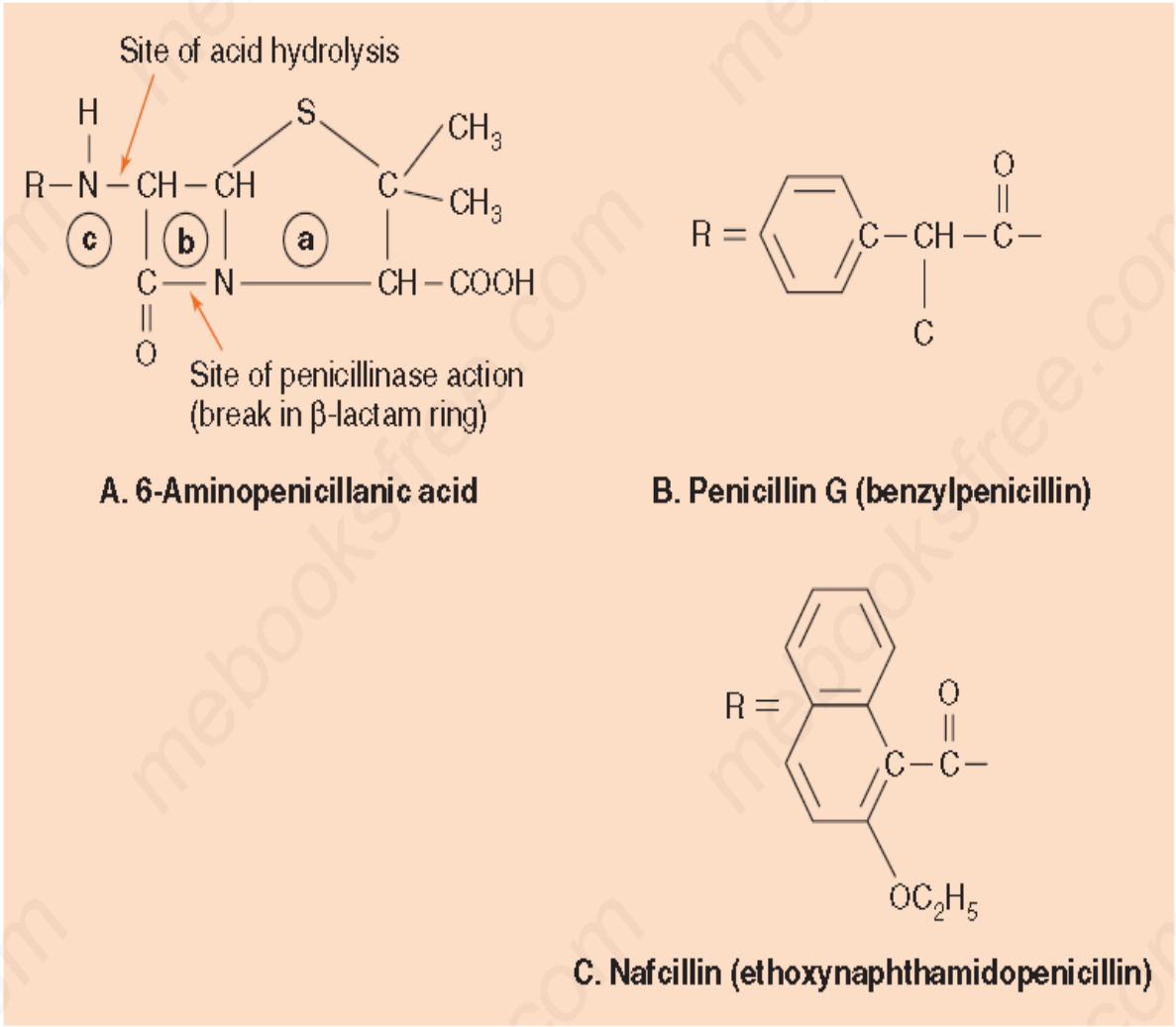


FIGURE 10–2 Penicillins. **A:** The 6-aminopenicillanic acid nucleus is composed of a thiazolidine ring (a), a β-lactam ring (b), and an amino group (c). The sites of inactivation by stomach acid and by penicillinase are indicated. **B:** The benzyl group, which forms benzylpenicillin (penicillin G) when attached at R. **C:** The large aromatic ring substituent that forms nafcillin, a β-lactamase–resistant penicillin, when attached at R. The large ring blocks the access of β-lactamase to the β-lactam ring.

TABLE 10–3 Activity of Selected Penicillins

Drug	Clinically Useful Activity[1]
Penicillin G	Gram-positive cocci, gram-positive rods, *Neisseria*, spirochetes such as *Treponema pallidum*, and many anaerobes (except *Bacteroides fragilis*) but none of the gram-negative rods listed below
Ampicillin or amoxicillin	Certain gram-negative rods, such as *Haemophilus influenzae*, *Escherichia coli*, *Proteus*, *Salmonella*, and *Shigella* but not *Pseudomonas aeruginosa*
Ticarcillin	*P. aeruginosa*, especially when used in synergistic combination with an aminoglycoside
Piperacillin	Similar to ticarcillin but with greater activity against *P. aeruginosa* and *Klebsiella pneumoniae*
Nafcillin or dicloxacillin	Penicillinase-producing *Staphylococcus aureus*

[1]The spectrum of activity is intentionally incomplete. It is simplified for the beginning student to illustrate the expanded coverage of gram-negative organisms with successive generations and does not cover all possible clinical uses.

- It can be seen that ampicillin and amoxicillin have activity against several gram-negative rods that the earlier penicillins do not have.
- These drugs are not useful against Pseudomonas aeruginosa and Klebsiella pneumoniae.
- The second important disadvantage—acid hydrolysis in the stomach—also has been addressed by modification of the side chain. The site of acid hydrolysis is the amide bond between the side chain and penicillanic acid nucleus

- The inactivation of penicillin G by β-lactamases is another important disadvantage, especially in the treatment of S. aureus infections.
- Access of the enzyme to the β-lactam ring is blocked by modification of the side chain with the addition of large aromatic rings containing bulky methyl or ethyl groups (**methicillin**, oxacillin, nafcillin, etc.;
- Another defense against β-lactamases is inhibitors such as clavulanic acid, tazobactam, sulbactam, and avibactam.
- Penicillins are usually nontoxic at clinically effective levels.
- The major disadvantage of these compounds is hypersensitivity, which is estimated to occur in 1% to 10% of patients.
- Patients who say they are allergic to penicillin can be treated with another equally effective antibiotic, if available.

Cephalosporins

- Cephalosporins are β-lactam drugs that act in the same manner as penicillins (i.e., they are bactericidal agents that inhibit the cross-linking of peptidoglycan).
- The structures, however, are different: Cephalosporins have a six-membered ring adjacent to the β-lactam ring and are substituted in two places on the 7-aminocephalosporanic acid nucleus
- whereas penicillins have a five-membered ring and are substituted in only one place.
- The first-generation cephalosporins are active primarily against gram-positive cocci

- These new cephalosporins have been categorized into second, third, and fourth generations, with each generation having expanded coverage against certain gram-negative rods.
- The fourth- and fifth-generation cephalosporins have activity against many gram-positive cocci as well.
- The inactivation of cephalosporins by β-lactamases (cephalosporinases) is an important clinical problem.

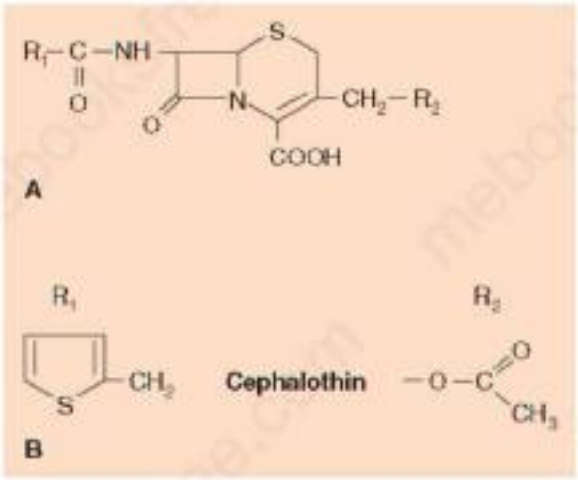


FIGURE 10–3 Cephalosporins. **A:** The 7-aminocephalosporanic acid nucleus. **B:** The two R groups in the drug cephalothin.

TABLE 10–4 Activity of Selected Cephalosporins[1]

Generation of Cephalosporin	Drug	Clinically Useful Activity
First	Cefazolin, cephalexin	Gram-positive cocci such as staphylococci and streptococci except enterococci and MRSA
Second	Cefuroxime	*Haemophilus influenzae*
	Cefoxitin	*Bacteroides fragilis*
Third	Ceftriaxone	Enteric gram-negative rods such as *Escherichia coli, Klebsiella* and *Proteus*. Also *Neisseria gonorrhoeae*
	Ceftazidime	*Pseudomonas aeruginosa* and other enteric gram-negative rods
Fourth	Cefepime	Enteric gram-negative rods that produce extended-spectrum β-lactamases; *Staphylococcus aureus* (not MRSA) and penicillin-resistant *Streptococcus pneumoniae*
Fifth	Ceftaroline	Gram-positive cocci and gram-negative rods that cause bacterial pneumonia and gram-positive cocci that cause skin infections including MRSA
	Ceftolozane	Enteric gram-negative rods that produce extended-spectrum β-lactamases; *Pseudomonas aeruginosa;* used in combination with tazobactam

MRSA = methicillin-resistant *Staphylococcus aureus*.

[1]The spectrum of activity is intentionally incomplete. It is simplified for the beginning student to illustrate the expanded coverage of gram-negative organisms with successive generations and does not cover all possible clinical uses.

- β-Lactamase inhibitors such as tazobactam and avibactam are combined with certain cephalosporins to prevent inactivation of the cephalosporin.
- For example, the FDA has approved the combination of ceftazidime/avibactam (Avycaz) and ceftolozane/tazobactam (Zerbaxa) for the treatment of intra-abdominal infections and complicated urinary tract infections caused by antibiotic-resistant gram-negative rods.

Carbapenems

- Carbapenems are β-lactam drugs that are structurally different from penicillins and cephalosporins.
- For example, imipenem (N-formimidoylthienamycin), a commonly used carbapenem, has a methylene group in the ring in place of the sulfur
- Imipenem has one of the widest spectrums of activity of the β-lactam drugs.
- It has excellent bactericidal activity against many gram-positive, gram negative, and anaerobic bacteria.

- It is effective against most gram-positive cocci (e.g., streptococci and staphylococci), most gram-negative cocci (e.g., Neisseria), many gram negative rods (e.g., Pseudomonas, Haemophilus, and members of the family Enterobacteriaceae such as E. coli), and various anaerobes (e.g., Bacteroides and Clostridium).
- Imipenem is especially useful in treating infections caused by gram-negative rods that produce extended-spectrum β-lactamases that make them resistant to all penicillins and cephalosporins.

- Carbapenems are often the “drugs of last resort” against bacteria resistant to multiple antibiotics.
- Imipenem is prescribed in combination with cilastatin, which is an inhibitor of dehydropeptidase, a kidney enzyme that inactivates imipenem.
- Imipenem is not inactivated by most β-lactamases; however, carbapenemases produced by K. pneumoniae that degrade imipenem and other carbapenemases have emerged.
- Other carbapenems, such as ertapenem and meropenem, are not inactivated by dehydropeptidase and are not prescribed in combination with cilastatin

Monobactams

- Monobactams are also β-lactam drugs that are structurally different from penicillins and cephalosporins.
- Monobactams are characterized by a β-lactam ring without an adjacent sulfur-containing ring structure (i.e., they are
- monocyclic).
- Aztreonam, currently the most useful monobactam, has excellent activity against many gram-negative rods, such as Enterobacteriaceae and Pseudomonas, but is inactive against gram-positive and anaerobic bacteria.

- It is resistant to most β-lactamases.
- It is very useful in patients who are hypersensitive to penicillin, because there is no cross-reactivity

Vancomycin

- Vancomycin is a glycopeptide that inhibits cell wall peptidoglycan synthesis by blocking transpeptidation but by a mechanism different from that of the β-lactam drugs.
- Vancomycin binds directly to the d-alanyl-d-alanine portion of the pentapeptide, which blocks the transpeptidase from binding, whereas the β-lactam drugs bind to the transpeptidase itself.
- Vancomycin also inhibits a second enzyme, the bacterial transglycosylase, which also functions in synthesizing the peptidoglycan.

- Vancomycin is a bactericidal agent effective against certain gram-positive bacteria.
- Its most important use is in the treatment of infections caused by S. aureus strains that are resistant to penicillinase-resistant penicillins such as nafcillin and methicillin (e.g., methicillin-resistant S. aureus [MRSA]).
- Note that vancomycin is not a β-lactam drug and, therefore, is not degraded by β-lactamase
- Vancomycin is also used in the treatment of infections caused by Staphylococcus epidermidis, penicillin-resistant Streptococcus pneumoniae, and enterococci.
- Strains of S. aureus, S. epidermidis, and enterococci with partial or complete resistance to vancomycin have been recovered from patients.

- A well-known adverse effect of vancomycin **is "red man"** syndrome. "Red" refers to the flushing caused by vasodilation
- induced by histamine release from mast cells and basophils
- **Telavancin** is a synthetic derivative of vancomycin that both inhibits peptidoglycan synthesis and disrupts bacterial cell membranes.
- It is used for the treatment of skin and soft tissue infections, especially those caused by MRSA

Cycloserine & Bacitracin

- Cycloserine is a structural analogue of d-alanine that inhibits the synthesis of the cell wall dipeptide d-alanyl-dalanine.
- It is used as a second-line drug in the treatment of tuberculosis.
- Bacitracin is a cyclic polypeptide antibiotic that prevents the dephosphorylation of the phospholipid that carries the peptidoglycan subunit across the cell membrane.
- This blocks the regeneration of the lipid carrier an inhibits cell wall synthesis.
- Bacitracin is a bactericidal drug useful in the treatment of superficial skin infections but too toxic for systemic use

2. Inhibition of Fungal Cell Wall Synthesis

- Echinocandins, such as caspofungin (Cancidas) and micafungin (Mycamine), are lipopeptides that block fungal cell wall synthesis by inhibiting the enzyme that synthesizes β-glucan.
- β-Glucan is a polysaccharide composed of long chains of d-glucose, which is an essential component of certain medically important fungal pathogens.
- Caspofungin inhibits the growth of Aspergillus and Candida but not Cryptococcus or Mucor.
- Caspofungin is used for the treatment of disseminated candidiasis and for the treatment of invasive aspergillosis that does not respond to amphotericin B.

- Micafungin is approved for the treatment of esophageal candidiasis and the prophylaxis of invasive Candida infections in bone marrow transplant patients.
- Anidulafungin is approved for the treatment of esophageal candidiasis and other serious Candida infections.

Inhibition of protein synthesis

- Several drugs inhibit protein synthesis in bacteria without significantly interfering with protein synthesis in human cells.
- Chloramphenicol, macrolides such as azithromycin and erythromycin, clindamycin, and linezolid act on the 50S subunit, whereas tetracyclines such as doxycycline and aminoglycosides such as gentamicin act on the 30S subunit.

1. Drugs That Act on the 30S Subunit Aminoglycosides

- Aminoglycosides are bactericidal drugs especially useful against many gram-negative rods.
- Certain aminoglycosides are used against other organisms (e.g., streptomycin is used in the multidrug therapy of tuberculosis, and gentamicin is used in combination with penicillin G against enterococci).
- Aminoglycosides are named for the amino sugar component of the molecule, which is connected by a glycosidic linkage to other sugar derivatives

- The two important modes of action of aminoglycosides have been documented best for streptomycin; other aminoglycosides
- probably act similarly.
- Both inhibition of the initiation complex and misreading of messenger RNA (mRNA) occur; the former is probably more important for the bactericidal activity of the drug.
- An initiation complex composed of a streptomycin-treated 30S subunit, a 50S subunit, and mRNA will not function—that is, no peptide bonds are formed, no polysomes are made, and a frozen "streptomycin monosome" results.

- Misreading of the triplet codon of mRNA so that the wrong amino acid is inserted into the protein also occurs in streptomycin-treated bacteria.
- The site of action on the 30S subunit includes both a ribosomal protein and the ribosomal RNA (rRNA).
- As a result of inhibition of initiation and misreading, membrane damage occurs and the bacterium dies.

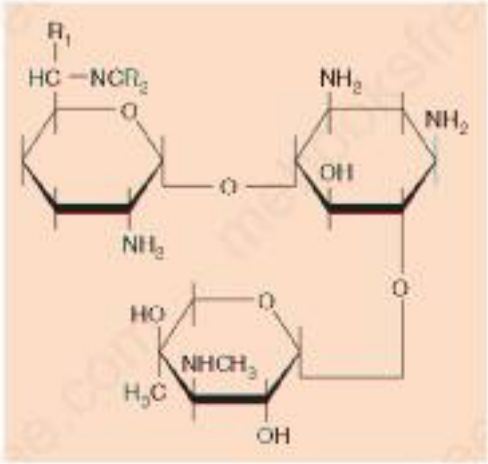


FIGURE 10–5 Aminoglycosides. Aminoglycosides consist of amino sugars joined by a glycosidic linkage. The structure of gentamicin is shown.

TABLE 10–5 Mode of Action of Antibiotics That Inhibit Protein Synthesis

Antibiotic	Ribosomal Subunit	Mode of Action	Bactericidal or Bacteriostatic
Aminoglycosides	30S	Blocks functioning of initiation complex and causes misreading of mRNA	Bactericidal
Tetracyclines	30S	Blocks tRNA binding to ribosome	Bacteriostatic
Chloramphenicol	50S	Blocks peptidyltransferase	Both[1]
Macrolides	50S	Blocks translocation	Primarily bacteriostatic
Clindamycin	50S	Blocks peptide bond formation	Primarily bacteriostatic
Linezolid	50S	Blocks early step in ribosome formation	Both[1]
Telithromycin	50S	Same as other macrolides (e.g., erythromycin)	Both[1]
Streptogramins	50S	Causes premature release of peptide chain	Both[1]

[1]Can be either bactericidal or bacteriostatic, depending on the organism.

TABLE 10–6 Spectrum of Activity of Antibiotics That Inhibit Protein Synthesis[1]

Antibiotic	Clinically Useful Activity	Comments
Aminoglycosides		
Streptomycin	Tuberculosis, tularemia, plague, brucellosis	Ototoxic and nephrotoxic
Gentamicin and tobramycin	Many gram-negative rod infections including *Pseudomonas aeruginosa*	Most widely used aminoglycosides
Amikacin	Same as gentamicin and tobramycin	Effective against some organisms resistant to gentamicin and tobramycin
Neomycin	Preoperative bowel preparation	Too toxic to be used systemically; use orally since not absorbed
Tetracyclines	Rickettsial and chlamydial infections, *Mycoplasma pneumoniae*	Not given during pregnancy or to young children
Tigecycline	Skin infections caused by various gram-positive cocci and intra-abdominal infections caused by various facultative and anaerobic bacteria (see text)	Adverse effects similar to tetracyclines
Chloramphenicol	*Haemophilus influenzae* meningitis, typhoid fever, anaerobic infections (especially *Bacteroides fragilis*)	Bone marrow toxicity limits use to severe infections
Macrolides	Pneumonia caused by *Mycoplasma* and *Legionella*, infections by gram-positive cocci in penicillin-allergic patients	Generally well tolerated but some diarrhea
Clindamycin	Anaerobes such as *Clostridium perfringens* and *B. fragilis*	Pseudomembranous colitis is a major side effect
Linezolid	Vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*, and penicillin-resistant pneumococci	Generally well tolerated
Telithromycin	Community-acquired pneumonia caused by various bacteria, including multidrug-resistant *Streptococcus pneumoniae*	Many bacteria that are resistant to other macrolides are susceptible to telithromycin
Streptogramins	Bacteremia caused by vancomycin-resistant *Enterococcus faecium*	No cross-resistance between streptogramins and other drugs that inhibit protein synthesis
Retapamulin	Skin infections caused by *Streptococcus pyogenes* and methicillin-sensitive *S. aureus*	

[1]The spectrum of activity is intentionally incomplete. It is simplified for the beginning student to illustrate the expanded coverage of gram-negative organisms with successive generations and does not cover all possible clinical uses.

Aminoglycosides have certain limitations in their use:

- (1) They have a toxic effect both on the kidneys and on the auditory and vestibular portions of the eighth cranial nerve.
  To avoid toxicity, serum levels of the drug, blood urea nitrogen, and creatinine should be measured.
- (2) They are poorly absorbed from the gastrointestinal tract and cannot be given orally.
- (3) They penetrate the spinal fluid poorly and must be given intrathecally in the treatment of meningitis.
- (4) They are ineffective against anaerobes, because their transport into the bacterial cell requires oxygen.

Tetracyclines

- Tetracyclines are a family of antibiotics with bacteriostatic activity against a variety of gram-positive and gram negative bacteria, mycoplasmas, chlamydiae, and rickettsiae.
- They inhibit protein synthesis by binding to the 30S ribosomal subunit and by blocking the aminoacyl transfer RNA (tRNA) from entering the acceptor site on the ribosome.
- The selective action of tetracycline on bacteria is not at the level of the ribosome, because tetracycline in vitro will inhibit protein synthesis equally well in purified ribosomes from both bacterial and human cells.
- Its selectivity is based on its greatly increased uptake into susceptible bacterial cells compared with human cells.

- In general, tetracyclines have low toxicity but are associated with some important side effects. One is suppression of the normal flora of the intestinal tract, which can lead to diarrhea and overgrowth by drug-resistant bacteria and fungi.
- Second is that suppression of Lactobacillus in the vaginal normal flora results in a rise in pH, which allows Candida albicans to grow and cause vaginitis.
- Third is brown staining of the teeth of fetuses and young children as a result of deposition of the drug in developing teeth; tetracyclines are avid calcium chelators
- For this reason, tetracyclines are contraindicated for use in pregnant women and in children younger than 8 years of age.

- Tetracyclines also chelate iron, and so products containing iron, such as iron-containing vitamins, should not be taken during therapy with tetracyclines.
- Tigecycline (Tygacil) is the first clinically available member of the glycylcycline class of antibiotics.
- They have a structure similar to tetracyclines and have the same mechanism of action as tetracyclines; namely, they bind to the 30S ribosomal subunit and inhibit bacterial protein synthesis.

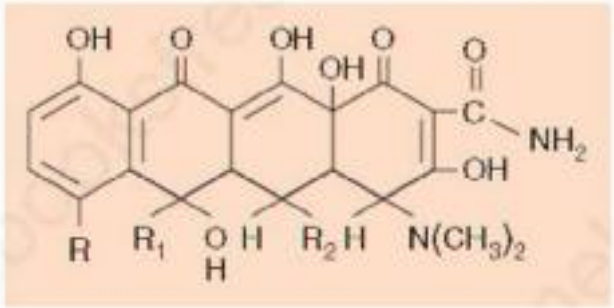


FIGURE 10–6 Tetracycline structure. The four-ring structure is depicted with its three R sites. Chlortetracycline, for example, has R = Cl, $R_1 = CH_3$, and $R_2 = H$.

2. Drugs That Act on the 50S Subunit

- **Chloramphenicol**
- Chloramphenicol is active against a broad range of organisms, including gram-positive and gram-negative bacteria (including anaerobes).
- It is bacteriostatic against certain organisms, such as Salmonella typhi, but has bactericidal activity against the three important encapsulated organisms that cause meningitis: Haemophilus influenzae, Streptococcus pneumoniae, and Neisseria meningitidis.
- Chloramphenicol inhibits protein synthesis by binding to the 50S ribosomal subunit and blocking the action of peptidyltransferase; this prevents the synthesis of new peptide bonds.

- It inhibits bacterial protein synthesis selectively, because it binds to the catalytic site of the transferase in the 50S bacterial ribosomal subunit but not to the transferase in the 60S human ribosomal subunit.
- Chloramphenicol inhibits protein synthesis in the mitochondria of human cells to some extent, since mitochondria have a 50S subunit (mitochondria are thought to have evolved from bacteria).
- This inhibition may be the cause of the dose dependent toxicity of chloramphenicol to bone marrow

- The most important side effect of chloramphenicol is bone marrow toxicity
- The other is aplastic anemia, which is caused by an idiosyncratic reaction to the drug
- One specific toxic manifestation of chloramphenicol is "gray baby" syndrome, in which the infant's skin appears gray and vomiting and shock occur.

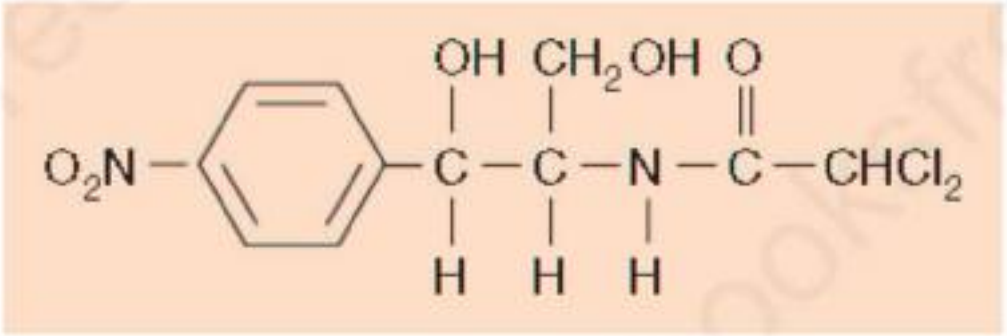


FIGURE 10–7 Chloramphenicol.

Macrolides

- Macrolides are a group of bacteriostatic drugs with a wide spectrum of activity. The name macrolide refers to their large (13–16 carbon) ring structure.
- **Azithromycin, erythromycin, and clarithromycin** are the main macrolides in clinical use.
- Azithromycin is used to treat genital tract infections caused by Chlamydia trachomatis and respiratory tract infections caused by Legionella, Mycoplasma, Chlamydia pneumoniae, and S. pneumoniae.
- Erythromycin has a similar spectrum of activity but has a shorter half-life and so must be taken more frequently and has more adverse effects, especially on the gastrointestinal tract.

- Clarithromycin is used primarily in the treatment of Helicobacter infections and in the treatment and prevention of Mycobacterium avium-intracellulare infections.
- An important adverse effect of clarithromycin is prolongation of the Q-T interval that may increase the risk of cardiac death.
- Macrolides inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit and blocking translocation.

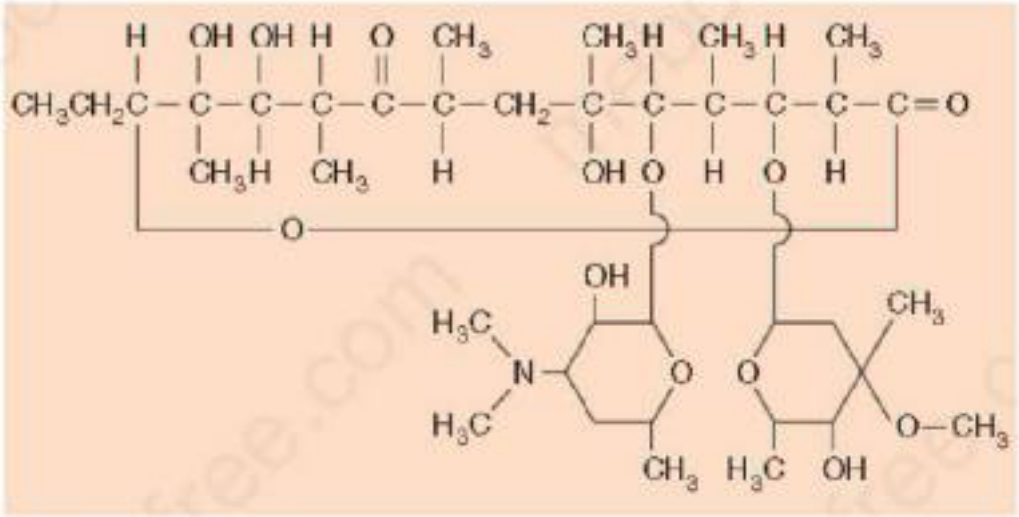


FIGURE 10–8 Erythromycin.

Clindamycin

- The most useful clinical activity of this bacteriostatic drug is against anaerobes, both gram-positive bacteria such as Clostridium perfringens and gram-negative bacteria such as B. fragilis.
- Clindamycin binds to the 50S subunit and blocks peptide bond formation by an undetermined mechanism.
- Its specificity for bacteria arises from its inability to bind to the 60S subunit of human ribosomes.

- The most important side effect of clindamycin is pseudomembranous colitis, which, in fact, can occur with virtually any antibiotic, whether taken orally or parenterally.
- The pathogenesis of this potentially severe complication is suppression of the normal flora of the bowel by the drug and overgrowth of a drug-resistant strain of C. difficile.
- The organism secretes an exotoxin that produces the pseudomembrane in the colon and severe, often bloody diarrhea

Linezolid

- Linezolid is useful for the treatment of vancomycin-resistant enterococci, methicillin-resistant S. aureus and S. epidermidis, and penicillin-resistant pneumococci.
- It is bacteriostatic against enterococci and staphylococci but bactericidal against pneumococci.
- Linezolid binds to the 23S ribosomal RNA in the 50S subunit and inhibits protein synthesis, but the precise mechanism is unknown.

- It appears to block some early step (initiation) in ribosome formation.
- Tedizolid is a second generation drug in the same class as linezolid but is approximately 10 times more effective.
- It is used for the treatment of skin and soft tissue infections caused by a similar range of bacteria as linezolid and has a similar mechanism of action.

Telithromycin

- Telithromycin (Ketek) is the first clinically useful member of the ketolide group of antibiotics.
- It is similar to the macrolides in general structure and mode of action but is sufficiently different chemically such that organisms resistant to macrolides may be sensitive to telithromycin.
- It has a wide spectrum of activity against a variety of gram-positive and gram-negative bacteria (including macrolide-resistant pneumococci) and is used in the treatment of community acquired pneumonia, bronchitis, and sinusitis.

Streptogramins

- A combination of two streptogramins, quinupristin, and dalfopristin (Synercid), is used for the treatment of bloodstream infections caused by vancomycin-resistant Enterococcus faecium (but not vancomycin-resistant Enterococcus faecalis).
- It is also approved for use in infections caused by Streptococcus pyogenes, penicillin-resistant S. pneumoniae, methicillin-resistant S. aureus, and methicillin resistant S. epidermidis.
- Streptogramins cause premature release of the growing peptide chain from the 50S ribosomal subunit.

Retapamulin

- Retapamulin (Altabax) is the first clinically available member of a new class of antibiotics called pleuromutilins.
- These drugs inhibit bacterial protein synthesis by binding to the 23S RNA of the 50S subunit and blocking attachment of the donor tRNA.
- Retapamulin is a topical antibiotic used in the treatment of skin infections, such as impetigo, caused by S. pyogenes and methicillin-sensitive S. aureus.

Inhibition of nucleic acid synthesis

- **1. Inhibition of Precursor Synthesis Sulfonamides**
- Either alone or in combination with trimethoprim, sulfonamides are useful in a variety of bacterial diseases such as urinary tract infections caused by E. coli, otitis media caused by S. pneumoniae or H. influenzae in children, shigellosis, nocardiosis, and chancroid.
- In combination, they are also the drugs of choice for two additional diseases, toxoplasmosis and Pneumocystis pneumonia
- The sulfonamides are a large family of bacteriostatic drugs that are produced by chemical synthesis.

- The mode of action of sulfonamides is to block the synthesis of tetrahydrofolic acid, which is required as a methyl donor in the synthesis of the nucleic acid precursors adenine, guanine, and thymine.
- Sulfonamides are structural analogues of p-aminobenzoic acid (PABA).
- PABA condenses with a pteridine compound to form dihydropteroic acid, a precursor of tetrahydrofolic acid
- Sulfonamides compete with PABA for the active site of the enzyme dihydropteroate synthetase. This competitive inhibition can be overcome by an excess of PABA.

- The basis of the selective action of sulfonamides on bacteria is that many bacteria synthesize their folic acid from PABA-containing precursors, whereas human cells require preformed folic acid as an exogenous nutrient because they lack the enzymes to synthesize it.

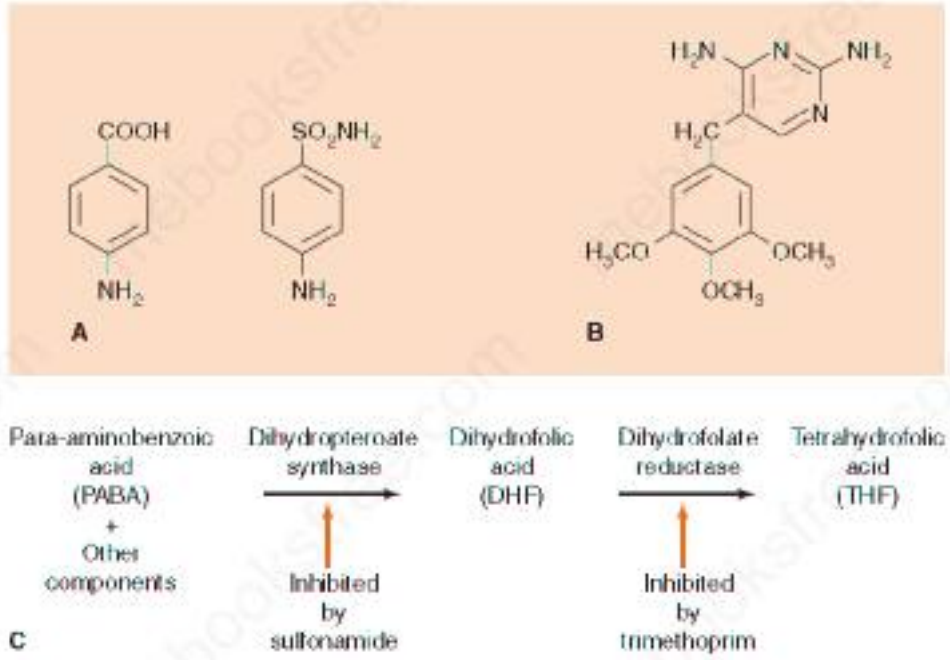


FIGURE 10–9 Mechanism of action of sulfonamides and trimethoprim. **A:** Comparison of the structures of *p*-aminobenzoic acid (PABA)

TABLE 10–7 Mode of Action and Activity of Selected Nucleic Acid Inhibitors[1]

Drug	Mode of Action	Clinically Useful Activity
Sulfonamides (e.g., sulfamethoxazole)	Inhibit folic acid synthesis; act as a competitive inhibitor of PABA	Used in combination with trimethoprim for UTI caused by *Escherichia coli*; otitis media and sinusitis caused by *Haemophilus influenzae*; MRSA; *Pneumocystis* pneumonia
Trimethoprim	Inhibits folic acid synthesis by inhibiting DHFR	Used in combination with sulfonamides for the uses described above
Fluoroquinolones (e.g., ciprofloxacin, levofloxacin)	Inhibit DNA synthesis by inhibiting DNA gyrase	Ciprofloxacin is used to treat GI tract infections caused by *Shigella* and *Salmonella*, and UTI caused by enteric gram-negative rods. Levofloxacin is used to treat respiratory tract infections, especially those caused by penicillin-resistant *Streptococcus pneumoniae*
Flucytosine	Inhibits thymidine synthesis by inhibiting thymidylate synthetase	Used in combination with amphotericin B for cryptococcal meningitis
Rifampin	Inhibits mRNA synthesis by inhibiting RNA polymerase	Used in combination with isoniazid and other drugs to treat tuberculosis

DHFR = dihydrofolate reductase; GI = gastrointestinal; MRSA = methicillin-resistant *Staphylococcus aureus*; PABA = para-aminobenzoic acid; UTI = urinary tract infection.

[1]The spectrum of activity is intentionally incomplete. It is simplified for the beginning student to emphasize the most common uses.

Trimethoprim

- Trimethoprim also inhibits the production of tetrahydrofolic acid but by a mechanism different from that of the sulfonamides
- (i.e., it inhibits the enzyme dihydrofolate reductase)
- Its specificity for bacteria is based on its much greater affinity for bacterial reductase than for the human enzyme.
- Trimethoprim is used most frequently together with sulfamethoxazole.
- Note that both drugs act on the same pathway—but at different sites—to inhibit the synthesis of tetrahydrofolate.

The advantages of the combination

- 1) bacterial mutants resistant to one drug will be inhibited by the other
- (2) the two drugs can act synergistically (i.e., when used together, they cause significantly greater inhibition than the sum of the inhibition caused by each drug separately).
- Trimethoprim-sulfamethoxazole is clinically useful in the treatment of urinary tract infections, Pneumocystis pneumonia, and shigellosis.
- It also is used for prophylaxis in granulopenic patients to prevent opportunistic infections.

2. Inhibition of DNA Synthesis Fluoroquinolones

- Fluoroquinolones are bactericidal drugs that block bacterial DNA synthesis by inhibiting DNA gyrase (topoisomerase).
- Fluoroquinolones, such as ciprofloxacin, levofloxacin, norfloxacin, ofloxacin, and others, are active against a broad range of organisms that cause infections of the lower respiratory tract, intestinal tract, urinary tract, and skeletal and soft tissues.
- **Nalidixic acid**, which is a quinolone but not a fluoroquinolone, is much less active and is used only for the treatment of urinary tract infections.

- Fluoroquinolones should not be given to pregnant women and children under the age of 18 years because they damage growing bone and cartilage.
- The Food and Drug Administration has issued a warning regarding the possibility of Achilles tendonitis and tendon rupture associated with fluoroquinolone use, especially in those over 60 years of age and in patients receiving corticosteroids, such as prednisone.
- Another important adverse effect of fluoroquinolones is peripheral neuropathy, the symptoms of which include pain, burning, numbness, or tingling in the arms or legs

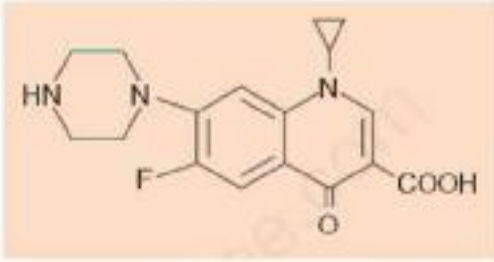


FIGURE 10–10 Ciprofloxacin. The triangle indicates a cyclopropyl group.

Flucytosine

- Flucytosine (5-fluorocytosine, 5-FC) is an antifungal drug that inhibits DNA synthesis.
- It is a nucleoside analogue that is metabolized to fluorouracil, which inhibits thymidylate synthetase, thereby limiting the supply of thymidine.
- It is used in combination with amphotericin B in the treatment of disseminated cryptococcal or candida infections, especially cryptococcal meningitis.
- It is not used alone because resistant mutants emerge very rapidly

3. inhibition of mRNA synthesis

- Rifampin is used primarily for the treatment of tuberculosis in combination with other drugs and for prophylaxis in close contacts of patients with meningitis caused by either N. meningitidis or H. influenzae.
- It is also used in combination with other drugs in the treatment of prosthetic-valve endocarditis caused by S. epidermidis.
- With the exception of the short-term prophylaxis of meningitis, rifampin is given in combination with other drugs because resistant mutants appear at a high rate when it is used alone.

- The selective mode of action of rifampin is based on blocking mRNA synthesis by bacterial RNA polymerase without affecting the RNA polymerase of human cells.
- Rifampin is red, and the urine, saliva, and sweat of patients taking rifampin often turn orange; this is disturbing but harmless.
- Rifampin is excreted in high concentration in saliva, which accounts for its success in the prophylaxis of bacterial meningitis since the organisms are carried in the throat.

- Rifabutin, a rifampin derivative with the same mode of action as rifampin, is useful in the prevention of disease caused by Mycobacterium avium-intracellulare in patients with severely reduced numbers of helper T cells (e.g., acquired immunodeficiency syndrome [AIDS] patients).
- Fidaxomicin (Dificid) inhibits the RNA polymerase of
- C. difficile.
- It is used in the treatment of pseudomembranous colitis and in preventing relapses of this disease.

Alteration of cell membrane function

- **1. Alteration of Bacterial Cell Membranes**
- Polymyxins are a family of polypeptide antibiotics of which the clinically most useful compound is polymyxin E (colistin).
- It is active against gram-negative rods, especially P. aeruginosa, Acinetobacter baumannii, and carbapenemase- producing Enterobacteriaceae.
- Polymyxins are cyclic peptides composed of 10 amino acids, 6 of which are diaminobutyric acid.
- The positively charged free amino groups act like a cationic detergent to disrupt the phospholipid structure of the cell membrane.

- Daptomycin is a cyclic lipopeptide that disrupts the cell membranes of gram-positive cocci.
- It is bactericidal for organisms such as S. aureus, S. epidermidis, S. pyogenes, Enterococcus faecalis, and E. faecium, including methicillin resistant strains of S. aureus and S. epidermidis, vancomycin- resistant strains of S. aureus, and vancomycin-resistant strains of E. faecalis and E. faecium.
- It is approved for use in complicated skin and soft tissue infections caused by these bacteria

2. Alteration of Fungal Cell Membranes

- Amphotericin B, the most important antifungal drug, is used in the treatment of a variety of disseminated fungal diseases.
- It is a polyene with a series of seven unsaturated double bonds in its macrolide ring structure (poly means many, and -ene is a suffix indicating the presence of double bonds;.
- It disrupts the cell membrane of fungi because of its affinity for ergosterol, a component of fungal membranes but not of bacterial or human cell membranes.
- Fungi resistant to amphotericin B have rarely been recovered from patient specimens.
- .

- **Nystatin** is another polyene antifungal agent, which, because of its toxicity, is used topically for infections caused by the yeast Candida
- Terbinafine blocks ergosterol synthesis by inhibiting squalene epoxidase.
- It is used in the treatment of dermatophyte infections of the skin, fingernails, and toenails.
- Azoles are antifungal drugs that act by inhibiting ergosterol synthesis.
- They block cytochrome P-450–dependent demethylation of lanosterol, the precursor of ergosterol.

- Fluconazole, ketoconazole, voriconazole, posaconazole, and itraconazole are used to treat systemic fungal diseases; clotrimazole and miconazole are used only topically because they are too toxic to be given systemically.
- Ketoconazole is useful in the treatment of blastomycosis, chronic mucocutaneous candidiasis, coccidioidomycosis, and skin infections caused by dermatophytes.
- Fluconazole is useful in the treatment of candidal and cryptococcal infections. Itraconazole is used to treat histoplasmosis and blastomycosis.

10–12 Fluconazole.

Additional drug mechanisms

1. Antibacterial Activity

- Isoniazid, or isonicotinic acid hydrazide (INH), is a bactericidal drug highly specific for Mycobacterium tuberculosis and other mycobacteria.
- It is used in combination with other drugs to treat tuberculosis and by itself to prevent tuberculosis in exposed persons. Because it penetrates human cells well, it is effective against the organisms residing within macrophages.
- INH inhibits mycolic acid synthesis, which explains why it is specific for mycobacteria and relatively nontoxic for humans.
- The drug inhibits a reductase required for the synthesis of the long-chain fatty acids called mycolic acids that are an essential constituent of mycobacterial cell walls

- **Metronidazole (Flagyl)** is bactericidal against anaerobic bacteria such as Bacteroides fragilis and Clostridium difficile and it is used to treat bacterial vaginosis.
- (It is also effective against certain protozoa such as Giardia and Trichomonas.)
- Metronidazole is a prodrug that is activated to the active compound within anaerobic bacteria by ferredoxin-mediated reduction of its nitro gr

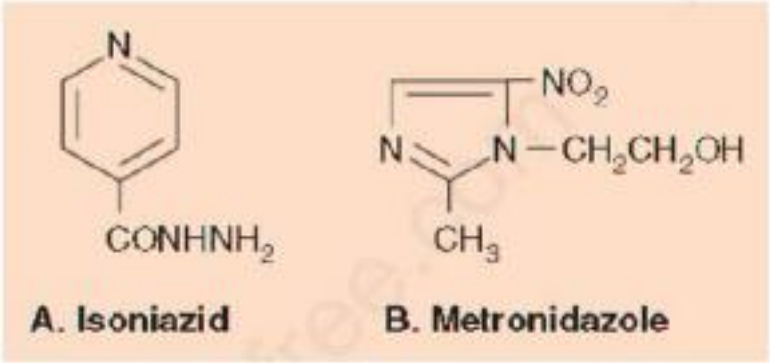


FIGURE 10–13 **A:** Isoniazid. **B:** Metronidazole.

- **Ethambutol** is a bacteriostatic drug used in the treatment of infections caused by M. tuberculosis and many of the atypical mycobacteria.
- It acts by inhibiting the synthesis of arabinogalactan, which functions as a link between the mycolic acids and the peptidoglycan of the organism.
- Pyrazinamide (PZA) is a bactericidal drug used in the treatment of tuberculosis but not in the treatment of most atypical mycobacterial infections.
- PZA is particularly effective against semidormant organisms in the lesion, which are not affected by INH or rifampin.

2. Antifungal Activity

- Griseofulvin is an antifungal drug that is useful in the treatment of hair and nail infections caused by dermatophytes.
- It binds to tubulin in microtubules and may act by preventing formation of the mitotic spindle.
- Pentamidine is active against fungi and protozoa.
- It is widely used to prevent or treat pneumonia caused by Pneumocystis jiroveci.

TABLE 10–8 Chemoprophylactic Use of Drugs Described in This Chapter

Drug	Use	Number of Chapter(s) for Additional Information
Penicillin	1. Prevent recurrent pharyngitis in high-risk patients who have had rheumatic fever	15
	2. Prevent syphilis in high-risk patients exposed to *Treponema pallidum*	24
	3. Prevent pneumococcal sepsis in splenectomized young children	15
Ampicillin	Prevent neonatal sepsis and meningitis in children born of mothers carrying group B streptococci	15
Amoxicillin	Prevent endocarditis caused by viridans streptococci in high-risk patients with damaged heart valves undergoing dental surgery	15
Cefazolin	Prevent staphylococcal surgical wound infections	15
Ceftriaxone	Prevent gonorrhea in high-risk patients exposed to *Neisseria gonorrhoeae*	16
Ciprofloxacin	1. Prevent meningitis in high-risk patients exposed to *Neisseria meningitidis*	16
	2. Prevent anthrax in high-risk patients exposed to *Bacillus anthracis*	17
	3. Prevent infection in neutropenic patients	68
Rifampin	Prevent meningitis in high-risk patients exposed to *N. meningitidis* and *Haemophilus influenzae*	16, 19
Isoniazid	Prevent progression of *Mycobacterium tuberculosis* in high-risk patients recently infected who are asymptomatic[1]	21
Erythromycin	1. Prevent pertussis in high-risk patients exposed to *Bordetella pertussis*	19
	2. Prevent gonococcal and chlamydial conjunctivitis in newborns	16, 25
Tetracycline	Prevent plague in high-risk patients exposed to *Yersinia pestis*	20
Fluconazole	Prevent cryptococcal meningitis in AIDS patients	50
Clotrimazole	Prevent thrush in AIDS patients and in others with reduced cell-mediated immunity	50
Trimethoprim-sulfamethoxazole	1. Prevent *Pneumocystis* pneumonia and *Toxoplasma* encephalitis in AIDS patients	52
	2. Prevent recurrent urinary tract infections	18
Pentamidine	Prevent *Pneumocystis* pneumonia in AIDS patients	52

AIDS = acquired immunodeficiency syndrome.

[1]Chemoprophylaxis with isoniazid is also viewed as treatment of asymptomatic individuals (see Chapter 21).

chemoprophylaxis.

- Some antibiotics are used to prevent diseases from occurring—a process called **chemoprophylaxis.**
- Chemoprophylaxis is used in three circumstances:
- Prior to surgery,
- In immunocompromised patients, and
- in people with normal immunity who have been exposed to certain pathogens.
- Of particular importance is the prevention of endocarditis in high-risk patients undergoing dental surgery by using amoxicillin perioperatively.

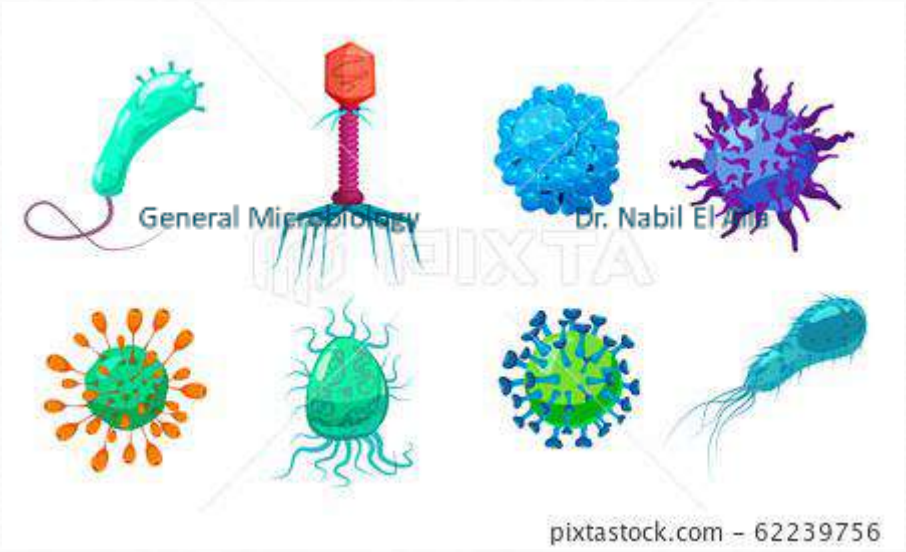
- High-risk patients include those who have a damaged heart valve or a prosthetic heart valve.
- Prophylaxis to prevent endocarditis in patients undergoing gastrointestinal or genitourinary tract surgery is not recommended.
- Cefazolin is often used to prevent staphylococcal infections
- in patients undergoing orthopedic surgery, including prosthetic joint implants, and in vascular graft surgery.
- Chemoprophylaxis is unnecessary in those with an implanted dialysis catheter, a cardiac pacemaker, or a ventriculoperitoneal shunt.

PROBIOTICS

- probiotics are live, nonpathogenic bacteria (or yeasts) that may be effective in the treatment or prevention of certain human diseases.
- The suggested basis for the possible beneficial effect lies either in providing colonization resistance by which the nonpathogen excludes the pathogen from binding sites on the mucosa, in enhancing the immune response against the pathogen, or in reducing the inflammatory response against the pathogen

- For example, the oral administration of live Lactobacillus rhamnosus strain GG significantly reduces the number of cases of nosocomial diarrhea in young children.
- Also, the yeast Saccharomyces boulardii reduces the risk of antibiotic associated diarrhea caused by C. difficile.
- Adverse effects are few; however, serious complications have arisen in highly immunosuppressed patients and in patients with indwelling vascular catheters.

End of Lecture



General Microbiology

Antimicrobial Drug resistance

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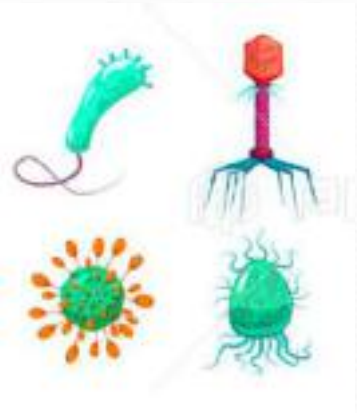


Four major mechanisms that mediate bacterial resistance to drugs

(1) Bacteria produce enzymes that inactivate the drug (e.g., β-lactamases can inactivate penicillins and cephalosporins by cleaving the β-lactam ring of the drug).

(2) Bacteria synthesize modified targets against which the drug has a reduced effect (e.g., a mutant protein in the 30S ribosomal subunit can result in resistance to streptomycin, and a methylated 23S rRNA can result in resistance to erythromycin).

(3) Bacteria reduce permeability to the drug such that an effective intracellular concentration of the drug is not achieved (e.g., changes in porins can reduce the amount of penicillin entering the bacterium).

- (4) Bacteria actively export drugs using a "multidrug-resistance pump" (MDR pump, or "efflux" pump). The MDR pump imports protons and, in an exchange-type reaction, exports a variety of foreign molecules including certain antibiotics, such as tetracyclines.
- Most drug resistance is due to a genetic change in the organism, either a chromosomal mutation or the acquisition of a plasmid or transposon.

TABLE 11–1 Mechanisms of Drug Resistance

Mechanism	Important Example	Drugs Commonly Affected
Inactivate drug	Cleavage by β-lactamase	β-Lactam drugs such as penicillins, cephalosporins
Modify drug target in bacteria	1. Mutation in penicillin-binding proteins	Penicillins
	2. Mutation in protein in 30S ribosomal subunit	Aminoglycosides, such as streptomycin
	3. Replace alanine with lactate in peptidoglycan	Vancomycin
	4. Mutation in DNA gyrase	Quinolones
	5. Mutation in RNA polymerase	Rifampin
	6. Mutation in catalase-peroxidase	Isoniazid
Reduce permeability of drug	Mutation in porin proteins	Penicillins, aminoglycosides, and others
Export of drug from bacteria	Multidrug-resistance pump	Tetracyclines, sulfonamides, quinolones

- High-level resistance refers to resistance that cannot be overcome by increasing the dose of the antibiotic.
- Low-level resistance refers to resistance that can be overcome by increasing the dose of the antibiotic.

- **Hospital-acquired infections** are significantly more likely to be caused by antibiotic-resistant organisms than are community-acquired infections.

- Hospital infections caused by Staphylococcus aureus and enteric gram-negative rods such as Escherichia coli and Pseudomonas aeruginosa.
- Antibiotic-resistant organisms are common in the hospital setting because widespread antibiotic use in hospitals selects for these organisms.
- Hospital strains are often resistant to multiple antibiotics.
- This resistance is usually due to the acquisition of plasmids carrying several genes that encode the enzymes that mediate resistance.

TABLE 11–2 Medically Important Bacteria That Exhibit Significant Drug Resistance

Type of Bacteria	Clinically Significant Drug Resistance
Gram-positive cocci	
Staphylococcus aureus	Penicillin G, methicillin/nafcillin
Streptococcus pneumoniae	Penicillin G
Enterococcus faecalis, E. faecium	Penicillin G, aminoglycosides, -vancomycin
Gram-negative cocci	
Neisseria gonorrhoeae	Penicillin G
Gram-positive rods	
None	
Gram-negative rods	
Haemophilus influenzae	Ampicillin
Pseudomonas aeruginosa	β-Lactams,[1] aminoglycosides
Enterobacteriaceae[2]	β-Lactams,[1] aminoglycosides
Mycobacteria	
Mycobacterium tuberculosis[3]	Isoniazid, rifampin
M. avium-intracellulare	Isoniazid, rifampin, and many others

Genetic basis of resistance

Chromosome-Mediated Resistance

- Chromosomal resistance is due to a mutation in the gene that codes for either the target of the drug or the transport system in the membrane that controls the uptake of the drug.
- The frequency of spontaneous mutations usually ranges from 10^{-7} to 10^{-9}, which is much lower than the frequency of acquisition of resistance plasmids.
- chromosomal resistance is less of a clinical problem than is plasmid-mediated resistance.

Plasmid-Mediated Resistance

Plasmid-mediated resistance is very important from a clinical point of view for three reasons:

1) It occurs in many different species, especially gram negative rods.
2) Plasmids frequently mediate resistance to multiple drugs.
3) Plasmids have a high rate of transfer from one cell to another, usually by conjugation.

- **Resistance plasmids (resistance factors, R factors)** are extrachromosomal, circular, double-stranded DNA molecules that carry the genes for a variety of enzymes that can degrade antibiotics and modify membrane transport systems
- R factors may carry one antibiotic resistance gene or may carry two or more of these genes.

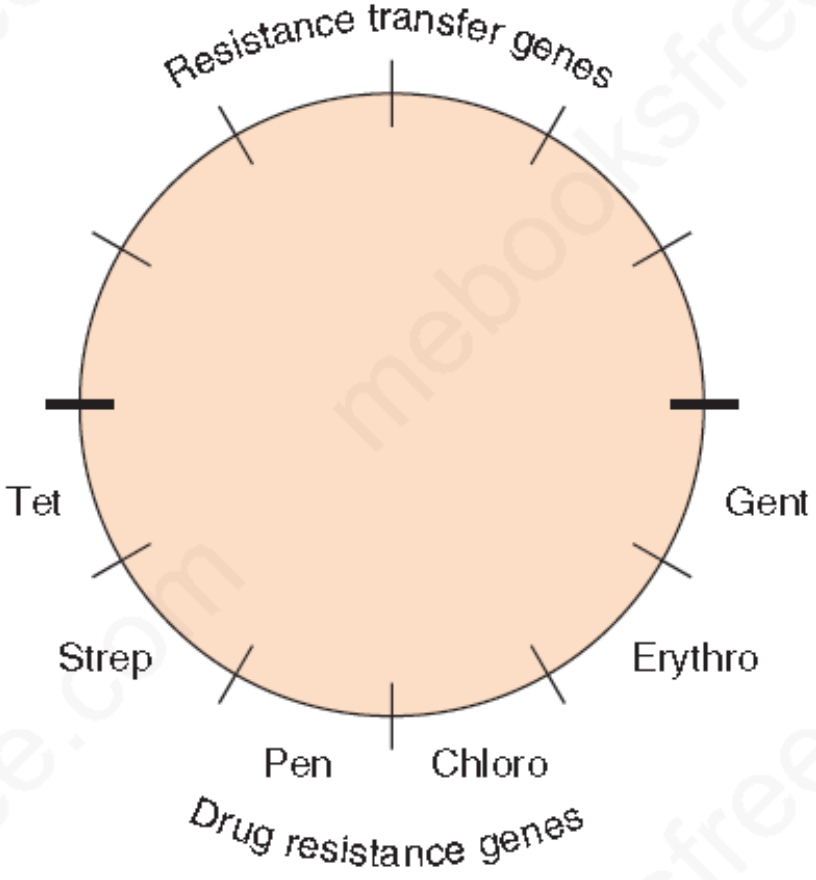


FIGURE 11–1 Resistance plasmid (R plasmid, R factor). Most resistance plasmids have two sets of genes: (1) resistance transfer genes that encode the sex pilus and other proteins that mediate transfer of the plasmid DNA during conjugation, and (2) drug resistance genes that encode the proteins that mediate drug resistance. The bottom half of the figure depicts (from left to right) the genes that encode resistance to tetracycline, streptomycin, penicillin (β-lactamase), chloramphenicol, erythromycin, and gentamicin.

- The medical implications of a plasmid carrying more than one resistance gene is twofold:
- First and most obvious is that a bacterium containing that plasmid can be resistant to more than one class of antibiotics (e.g., penicillins and aminoglycosides)
- Second, that the use of an antibiotic that selects for an organism resistant to one antibiotic will select for an organism that is resistant to all the antibiotics whose resistance genes are carried by the plasmid.
- For example, if an organism has the R plasmid, then the use of penicillin will select for an organism resistant not only to penicillin, but also to tetracyclines, aminoglycosides (e.g., streptomycin and gentamicin), chloramphenicol, and erythromycin.

TABLE 11–3 R-Factor–Mediated Resistance Mechanisms

Drug	Mechanism of Resistance
Penicillins and cephalosporins	β-Lactamase cleavage of β-lactam ring
Aminoglycosides	Modification by acetylation, adenylylation, or phosphorylation
Chloramphenicol	Modification by acetylation
Erythromycin	Change in receptor by methylation of rRNA
Tetracycline	Reduced uptake or increased export
Sulfonamides	Active export out of the cell and reduced affinity of enzyme

- R factors have two very important properties:

(1) they can replicate independently of the bacterial chromosome; therefore, a cell can contain many copies

(2) they can be transferred not only to cells of the same species, but also to other species and genera.

- The conjugal transfer is under the control of the genes of the R plasmid and not of the F (fertility) plasmid, which governs the transfer of the bacterial chromosome

R factors impart two other traits:

1) Resistance to metal ions (e.g., they code for an enzyme that reduces mercuric ions to elemental mercury)
2) Resistance to certain bacterial viruses by coding for restriction endonucleases that degrade the DNA of the infecting bacteriophages.

Transposon-Mediated Resistance

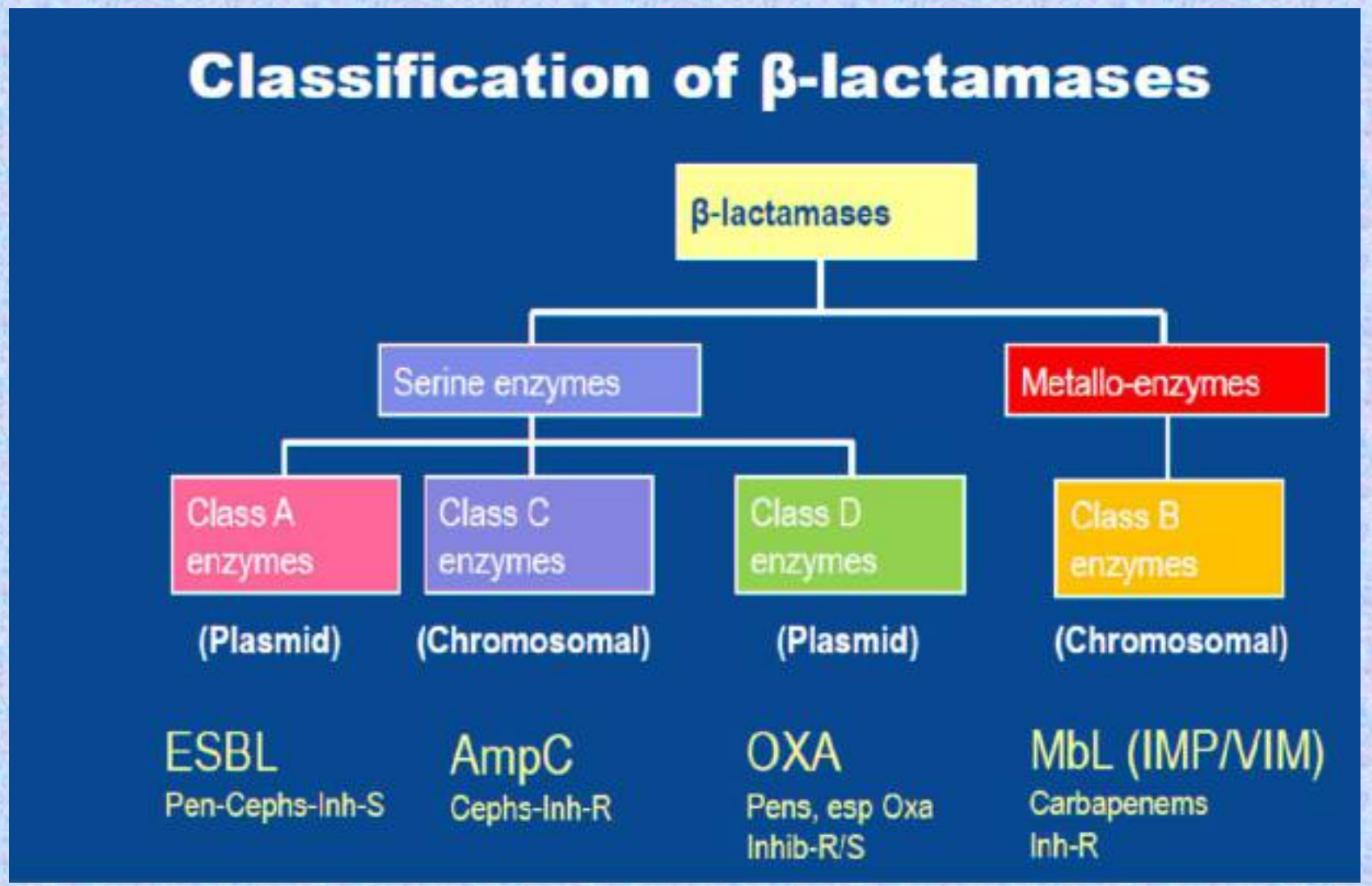
- Transposons are genes that are transferred either within or between larger pieces of DNA such as the bacterial chromosome and plasmids.
- A typical drug resistance transposon is composed of three genes flanked on both sides by shorter DNA sequences, usually a series of inverted repeated bases
- **The three genes code for :**
- (1) transposase, the enzyme that catalyzes excision and reintegration of the transposon;
- (2) a repressor that regulates the synthesis of the transposase;
- (3) the drug resistance gene.

Specific mechanisms of resistance

- **Penicillins & Cephalosporins**
- Cleavage by a-lactamases (penicillinases and cephalosporinases) is by far the most important .
- β-Lactamases produced by various organisms have different properties.
- staphylococcal penicillinase is inducible by penicillin and is secreted into the medium.
- some β-lactamases produced by several gram-negative rods are constitutively produced, are located in the periplasmic space near the peptidoglycan, and are not secreted into the medium.
- .

- Clavulanic acid, tazobactam, sulbactam, and avibactam are penicillin analogues that bind strongly to β-lactamases and inactivate them
- Combinations of these inhibitors and penicillins (e.g., clavulanic acid and amoxicillin [Augmentin]) can overcome resistance mediated by many but not all β-lactamases

Classification of β-lactamases

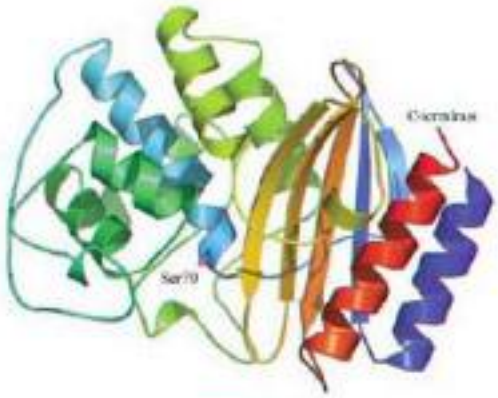


Extended-spectrum a-lactamases (ESBLs)

- Extended-spectrum a-lactamases (ESBLs) are produced by several enteric bacteria, notably E. coli, Klebsiella, Enterobacter, and Proteus.
- ESBLs endow the bacteria with resistance to all penicillins, cephalosporins, and monobactams.
- These bacteria remain sensitive to combinations such as piperacillin/tazobactam.
- In 2009, a new strain of highly resistant Klebsiella was isolated in India carrying a plasmid that encoded New Delhi metallo-alactamase (NDM-1).

- This plasmid confers high-level resistance to many antibiotics and has spread from Klebsiella to other member of the Enterobacteriaceae.
- Resistant Enterobacteriaceae carrying NDM-1 have emerged in many countries.

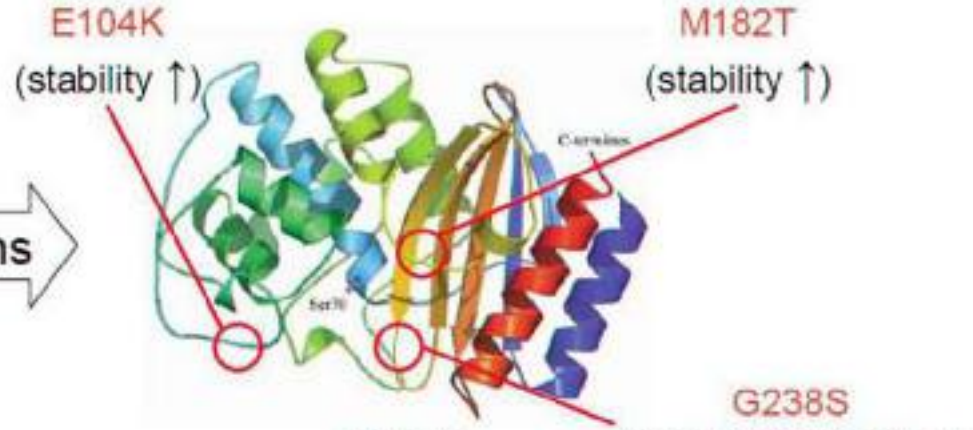
β-Lactamases



TEM-1

mutations

ESBLs



TEM-52

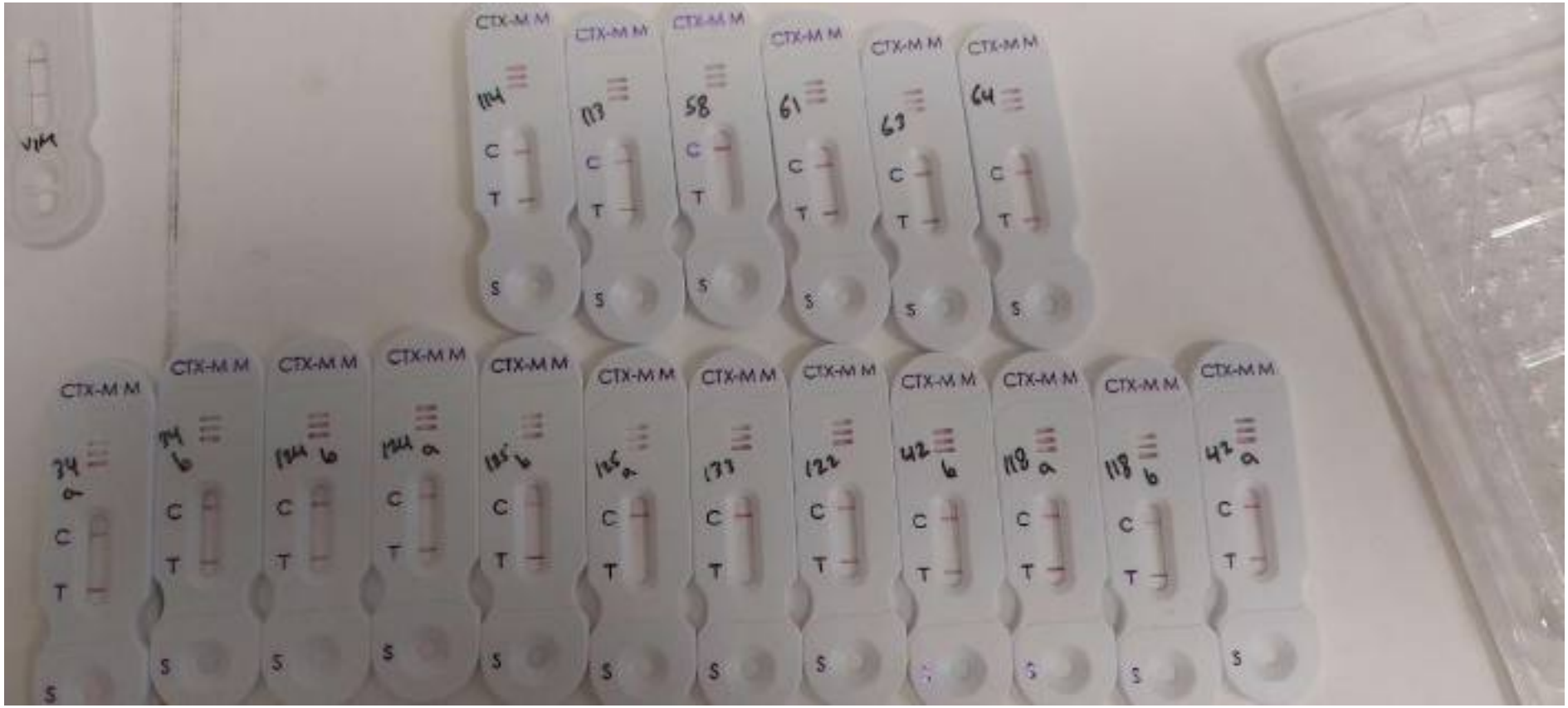
Spectrum:

- penicillins
- 1st generation cephalosporins
- 2nd generation cephalosporins

Extended Spectrum:

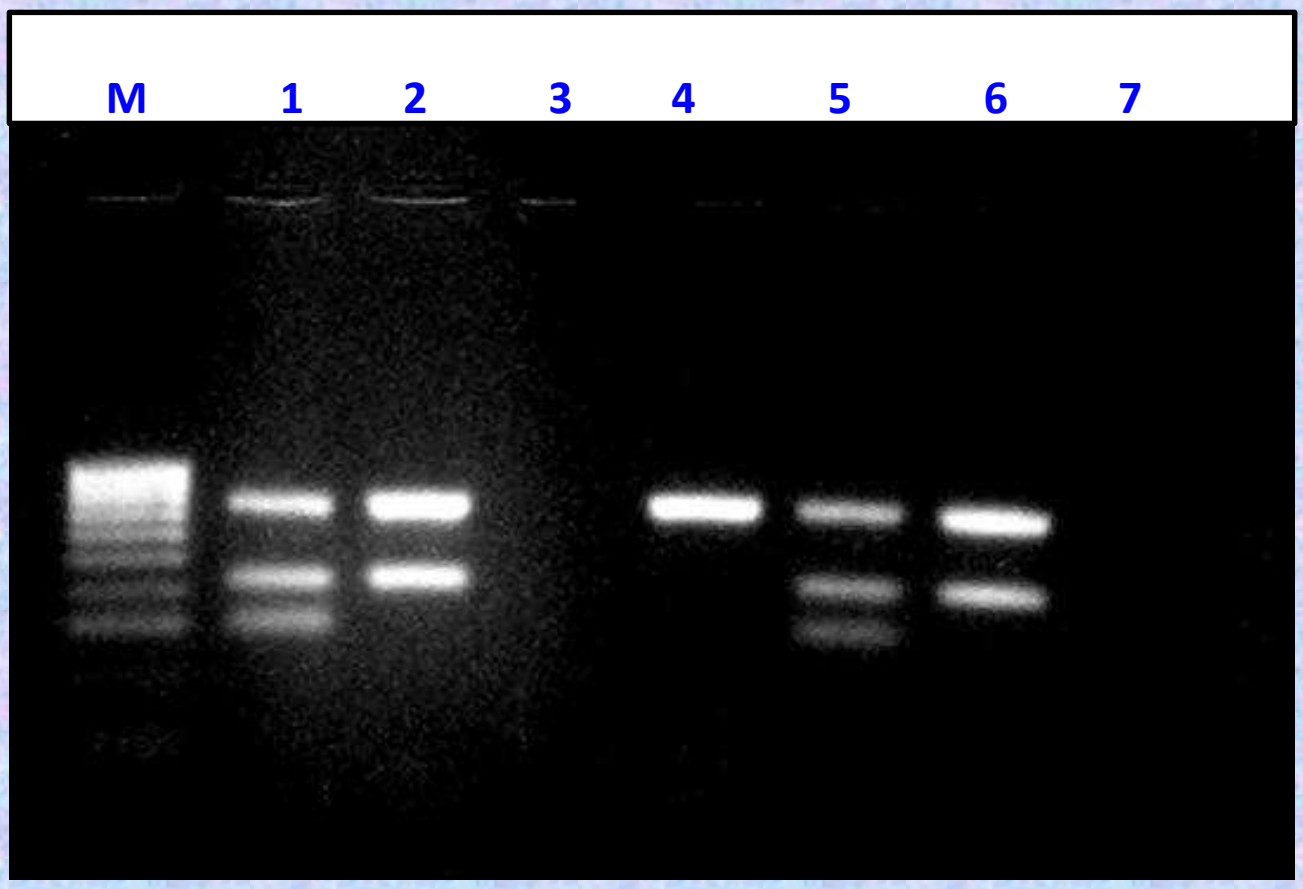
- penicillins
- 1st generation cephalosporins
- 2nd generation cephalosporins
- **3rd generation cephalosporins (e.g. cefotaxime)**
- **monobactams (e.g. aztreonam)**

Susceptible to cephamycins (cefotetan, cefoxitin), carbapenems (e.g. imipenem), and inhibitors



lateral flow immunoassay for the rapid identification of CTX-M

- Resistance to penicillins can also be due to changes in the penicillin-binding proteins (PBPs) in the bacterial cell membrane.
- These changes account for both the low-level and high-level resistance exhibited by S. pneumoniae to penicillin G and for the resistance of S. aureus to nafcillin and other β-lactamase–resistant penicillins.
- The resistance of MRSA to almost all β-lactams is attributed to the presence of PBP2a, which is found particularly in MRSA.
- The relative resistance of Enterococcus faecalis to penicillins may be due to altered penicillin-binding proteins.



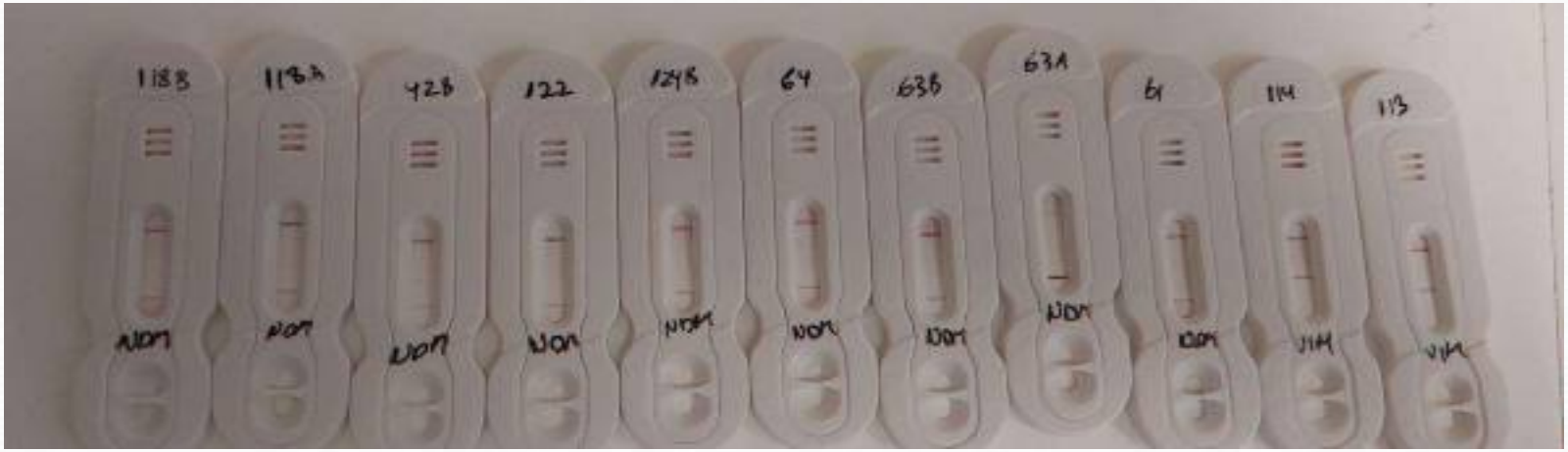
A representative result of MRSA PCR Amplicon;
lane M: 100 b DNA ladder; lane 1: positive control; Lane 3 Negative control, lanes 2: (16SrRNA + Mec A) (MRSA), lane 4: (16SrRNA) (MSSA), Lane 5: (16SrRNA + Mec A+ PVL gene) (MRSA+PVL), Lane 6: (16SrRNA + Mec A) and Lane 7 : BlanK

- Low-level resistance of N. gonorrhoeae to penicillin is attributed to poor permeability to the drug.
- High-level resistance is due to the presence of a plasmid coding for penicillinase.
- Some isolates of S. aureus demonstrate yet another form of resistance, called tolerance, in which growth of the organism is inhibited by penicillin but the organism is not killed.
- This is attributed to a failure of activation of the autolytic enzymes, murein hydrolases, which degrade the peptidoglycan.

Carbapenems

- Resistance to carbapenems, such as imipenem, is caused by carbapenemases that degrade the β-lactam ring.
- This enzyme endows the organism with resistance to penicillins and cephalosporins as well.
- **Carbapenemases** are produced by many enteric gram-negative rods, especially Klebsiella, Escherichia, and Pseudomonas.
- **Carbapenem-resistant strains of Klebsiella pneumoniae are an important cause of hospital-acquired infections** and are resistant to almost all known antibiotics.

lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48



Vancomycin

- Resistance to vancomycin is caused by a change in the peptide component of peptidoglycan from **d-alanyl-d-alanine**, which is the normal binding site for vancomycin, to **d-alanine- d-lactate**, to which the drug does not bind.
- Of the four gene loci mediating vancomycin resistance, VanA is the most important.
- It is carried by a transposon on a plasmid and provides high-level resistance to both vancomycin and teicoplanin.
- The VanA locus encodes those enzymes that synthesize d-alanine-d-lactate as well as several regulatory proteins.

Vancomycin-resistant strains of enterococci (VRE)

- VRE have been recovered from clinical specimens.
- Rare isolates of S. aureus that exhibit resistance to vancomycin have also been recovered from patient specimens.
- Rare isolates of S. pneumoniae that exhibit tolerance to vancomycin have been recovered as well.

Aminoglycosides

- **Resistance to aminoglycosides occurs by three mechanisms:**
- (1) modification of the drugs by plasmid-encoded phosphorylating, adenylylating, and acetylating enzymes (the most important mechanism);
- (2) chromosomal mutation (e.g., a mutation in the gene that codes for the target protein in the 30S subunit of the bacterial ribosome);
- (3) decreased permeability of the bacterium to the drug.

Tetracyclines

- Tetracyclines—Resistance to tetracyclines is the result of failure of the drug to reach an inhibitory concentration inside the bacteria.
  - This is due to plasmid-encoded processes that either reduce the uptake of the drug or enhance its transport out of the cell.

Chloramphenicol

- Resistance to chloramphenicol is due to a plasmid-encoded acetyltransferase that acetylates the drug, thus inactivating it.

Erythromycin

- Resistance to erythromycin is due primarily to a plasmid-encoded enzyme that methylates the 23S rRNA, thereby blocking binding of the drug.
- An efflux pump that reduces the concentration of erythromycin within the bacterium causes low-level resistance to the drug.
- An esterase produced primarily by enteric gram negative rods cleaves the macrolide ring, which inactivates the drug.

Sulfonamides

Resistance to sulfonamides is mediated primarily by two mechanisms:

- (1) a plasmid-encoded transport system that actively exports the drug out of the cell,
- (2) a chromosomal mutation in the gene coding for the target enzyme dihydropteroate synthetase, which reduces the binding affinity of the drug.

- **Trimethoprim**—Resistance to trimethoprim is due primarily to mutations in the chromosomal gene that encodes dihydrofolate reductase, the enzyme that reduces dihydrofolate to tetrahydrofolate.
- **Quinolones**—Resistance to quinolones is due primarily to chromosomal mutations that modify the bacterial DNA gyrase.

Rifampin

- Resistance to rifampin is due to a chromosomal mutation in the gene encoding the bacterial RNA polymerase, resulting in ineffective binding of the drug.
- Because resistance occurs at high frequency (10^{-5}), rifampin is not prescribed alone for the treatment of infections.
- It is used alone for the prevention of certain infections because it is administered for only a short time

- **Isoniazid—**Resistance of *M. tuberculosis* to isoniazid is due to mutations in the organism's catalase–peroxidase gene.
- Catalase or peroxidase enzyme activity is required to synthesize the metabolite of isoniazid that actually inhibits the growth of *M. tuberculosis*.
- **Ethambutol—**Resistance of *M. tuberculosis* to ethambutol is due to mutations in the gene that encodes arabinosyl transferase, the enzyme that synthesizes the arabinogalactan in the organism's cell wall.

- **Pyrazinamide**—Resistance of *M. tuberculosis* to pyrazinamide (PZA) is due to mutations in the gene that encodes bacterial amidase, the enzyme that converts PZA to the active form of the drug, pyrazinoic acid.

Nongenetic basis of resistance

- (1) Bacteria can be walled off within an abscess cavity that the drug cannot penetrate effectively. Surgical drainage is therefore a necessary adjunct to chemotherapy.
- (2) Bacteria can be in a resting state (i.e., not growing); they are therefore insensitive to cell wall inhibitors such as penicillins and cephalosporins.
- (3) Under certain circumstances, organisms that would ordinarily be killed by penicillin can lose their cell walls, survive as protoplasts, and be insensitive to cell wall–active drugs.
- Later, if such organisms resynthesize their cell walls, they are fully susceptible to these drugs.

- (4) The presence of foreign bodies makes successful antibiotic treatment more difficult.
- This applies to foreign bodies such as surgical implants and catheters as well as materials that enter the body at the time of penetrating injuries, such as splinters and shrapnel.
- (5) Several artifacts can make it appear that the organisms are resistant (e.g., administration of the wrong drug or the wrong dose or failure of the drug to reach the appropriate site in the body).

Three main points of overuse and misuse of antibiotics

- (1) Some physicians use multiple antibiotics when one would be sufficient, prescribe unnecessarily long courses of antibiotic therapy, use antibiotics in self-limited infections for which they are not needed, and overuse antibiotics for prophylaxis before and after surgery.
- (2) In many countries, antibiotics are sold over the counter to the general public; this practice encourages inappropriate and indiscriminate use of the drugs.
- (3) Antibiotics are used in animal feed to prevent infections and promote growth. This selects for resistant organisms in the animals and may contribute to the pool of resistant organisms in humans.

Antibiotic sensitivity testing

Antibiogram

- An antibiogram is the term used to describe the results of antibiotic susceptibility tests performed on the bacteria isolated from the patient.
- Other factors such as the patient's renal function and hypersensitivity profile must also be considered in choosing the antibiotic.
- There are two types of tests used to determine the antibiogram:

(1) the tube dilution test that determines the minimal inhibitory concentration

- (2) the disk diffusion (Kirby-Bauer) test that determines the diameter of the zone of inhibition

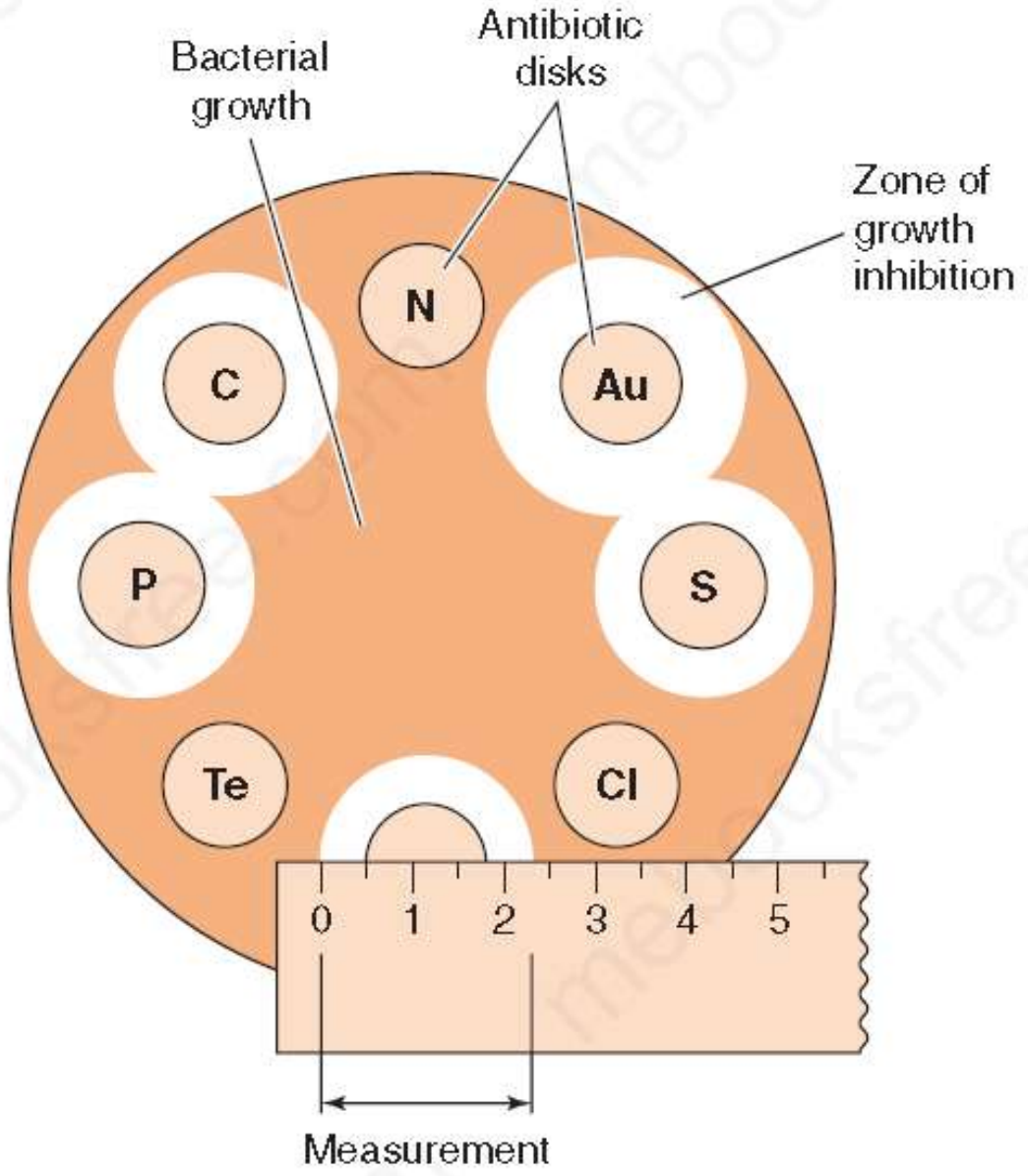


FIGURE 11–3 Antibiotic sensitivity testing. A zone of inhibi-
tion surrounds several antibiotic containing disks. A zone of certain

Minimal Inhibitory Concentration

- Minimal inhibitory concentration (MIC), which is defined as the lowest concentration of drug that inhibits the growth of the organism.
- The MIC is determined by inoculating the organism isolated from the patient into a series of tubes or cups containing twofold dilutions of the drug.
- After incubation at 35°C for 18 hours, the lowest concentration of drug that prevents visible growth of the organism is the MIC.
- This provides the physician with a precise concentration of drug to guide the choice of both the drug and the dose.

- A second method of determining antibiotic sensitivity is the disk diffusion method, in which disks impregnated with various antibiotics are placed on the surface of an agar plate that has been inoculated with the organism isolated from the patient
- After incubation at 35°C for 18 hours, during which time the antibiotic diffuses outward from the disk, the diameter of the zone of inhibition is determined.
- The size of the zone of inhibition is compared with standards to determine the sensitivity of the organism to the drug.

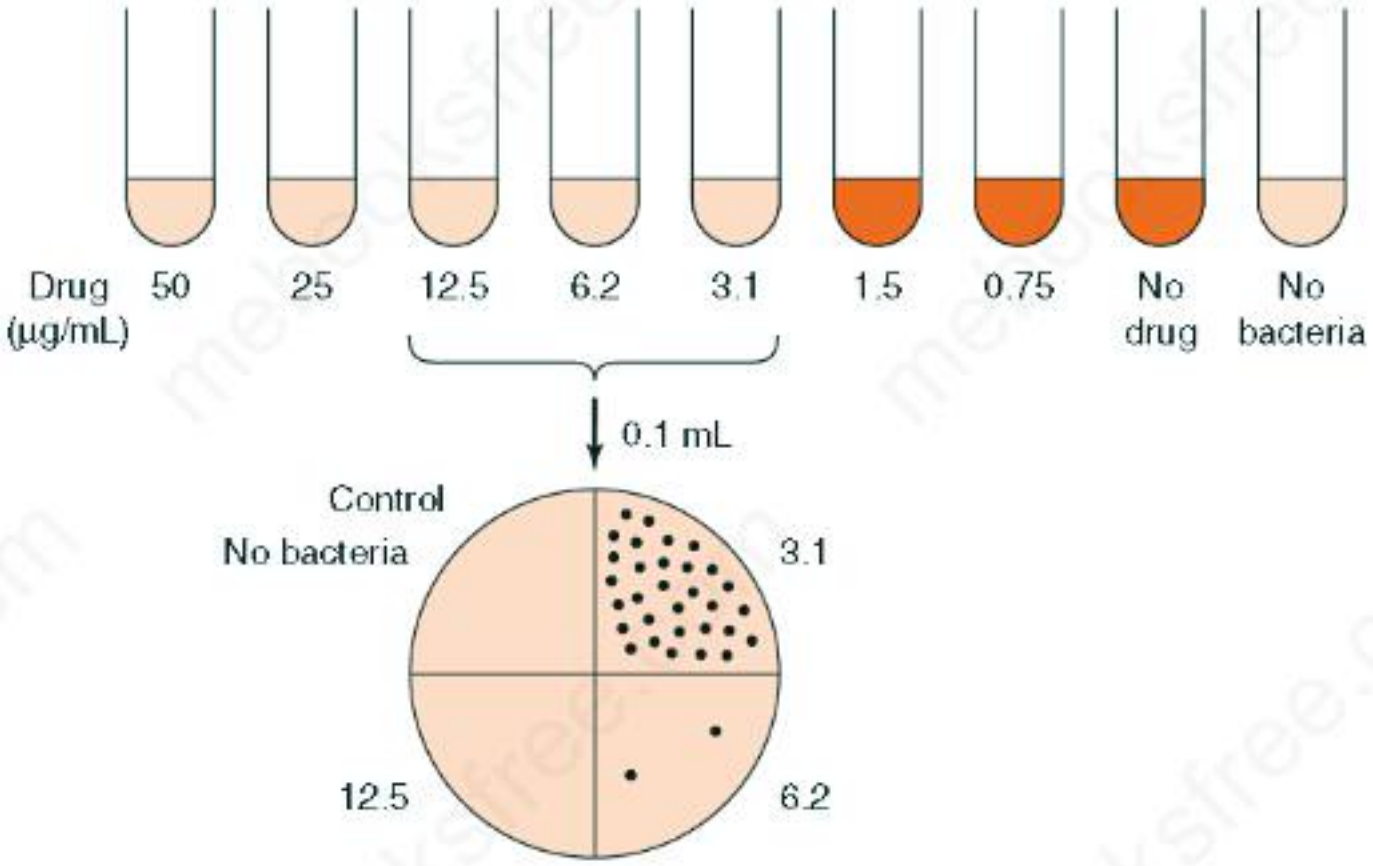


FIGURE 11–2 Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

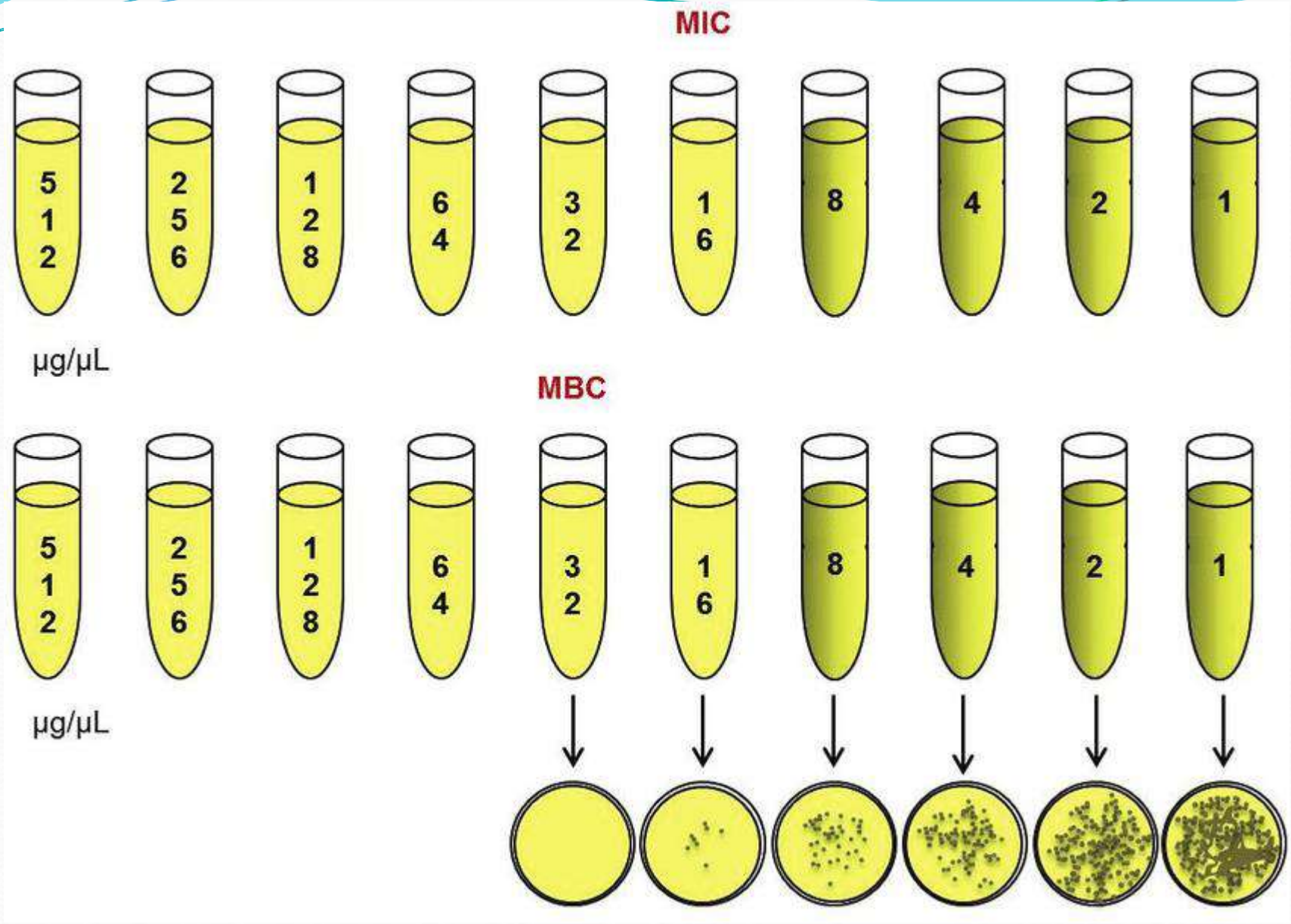
Minimal Bactericidal Concentration

- For certain infections, such as endocarditis, it is important to know the concentration of drug that actually kills the organism rather than the concentration that merely inhibits growth.

This concentration, called the minimal bactericidal concentration (MBC),

- is determined by taking a small sample (0.01 or 0.1 mL) from the tubes used for the MIC assay and spreading it over the surface of a drug-free blood agar plate

- Any organisms that were inhibited but not killed now have a chance to grow because the drug has been diluted significantly. After incubation at 35°C for 48 hours, the lowest concentration that has reduced the number of colonies by 99.9%, compared with the drug-free control, is the MBC.
- Bactericidal drugs usually have an MBC equal or very similar to the MIC, whereas bacteriostatic drugs usually have an MBC significantly higher than the MIC.


MIC
512
256
128
64
32
16
8
4
2
1
µg/µL
MBC
512
256
128
64
32
16
8
4
2
1
µg/µL

Serum Bactericidal Activity

- In the treatment of endocarditis, it can be useful to determine whether the drug is effective by assaying the ability of the drug in the patient's serum to kill the organism.
- This test, called the serum bactericidal activity, is performed in a manner similar to that of the MBC determination, except that it is a serum sample from the patient, rather than a standard drug solution, that is used.
- After a standard inoculum of the organism has been added and the mixture has been incubated at 35°C for 18 hours, a small sample is subcultured onto blood agar plates, and the serum dilution that kills 99.9% of the organisms is determined.
- Clinical experience has shown that a peak1 serum bactericidal activity of 1:8 or 1:16 is adequate for successful therapy of endocarditis

Beta-Lactamase Production

- For severe infections caused by certain organisms, such as S. aureus and Haemophilus influenzae, it is important to know as soon as possible whether the organism isolated from the patient is producing β-lactamase.
- Rapid assays for the enzyme can be used that yield an answer in a few minutes, as opposed to an MIC test or adisk diffusion test, both of which take 18 hours.
- A commonly used procedure is the chromogenic β-lactam method, in which a colored β-lactam drug is added to a suspension of the organisms.
- If β-lactamase is made, hydrolysis of the β-lactam ring causes the drug to turn a different color in 2 to 10 minutes.
- Disks impregnated with a chromogenic β-lactam can also be used.

Use of antibiotic combinations

- (1) To treat serious infections before the identity of the
- organism is known.
- (2) To achieve a synergistic inhibitory effect against certain organisms.
- (3) To prevent the emergence of resistant organisms. (If bacteria become resistant to one drug, the second drug will kill them, thereby preventing the emergence of resistant strains.)

Two drugs can interact in one of several ways

- They are usually indifferent to each other (i.e., additive only).
- Sometimes there is a synergistic interaction, in which the effect of the two drugs together is significantly greater than the sum of the effects of the two drugs acting separately.
- Rarely, the effect of the two drugs together is antagonistic, in which the result is significantly lower activity than the sum of the activities of the two drugs alone.

- A synergistic effect can result from a variety of mechanisms.

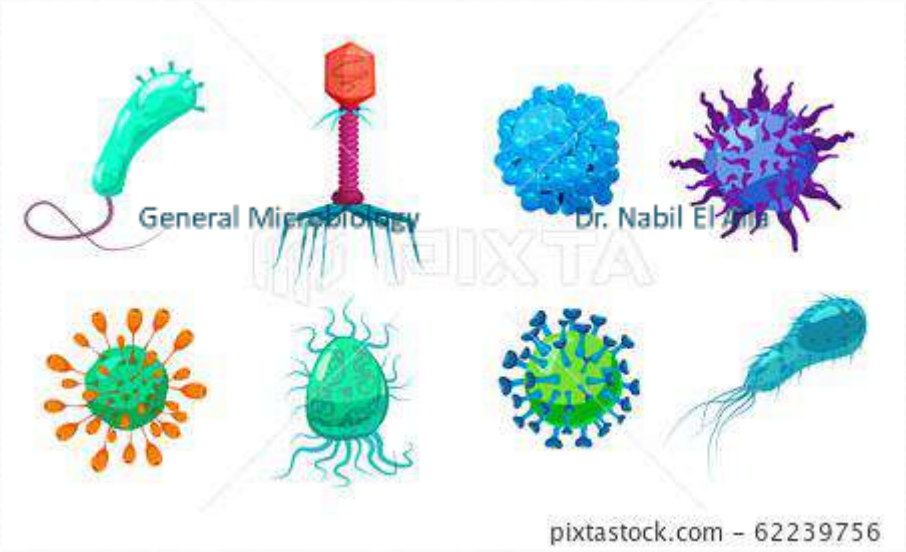
For example, the combination of a penicillin and an aminoglycoside such as gentamicin has a synergistic action against enterococci (E. faecalis), because penicillin damages the cell wall sufficiently to enhance the entry of aminoglycoside.

When given alone, neither drug is effective.

- A second example is the combination of a sulfonamide with trimethoprim. In this instance, the two drugs act on the same metabolic pathway, such that if one drug does not inhibit folic acid synthesis sufficiently, the second drug provides effective inhibition by blocking a subsequent step in the pathway.

- Although antagonism between two antibiotics is unusual, one example is clinically important.
- This involves the use of penicillin G combined with the bacteriostatic drug tetracycline in the treatment of meningitis caused by S. pneumoniae.
- Antagonism occurs because tetracycline inhibits the growth of the organism, thereby preventing the bactericidal effect of penicillin G, which kills only growing organisms.

End of Lecture



General Microbiology

Bacterial Vaccines

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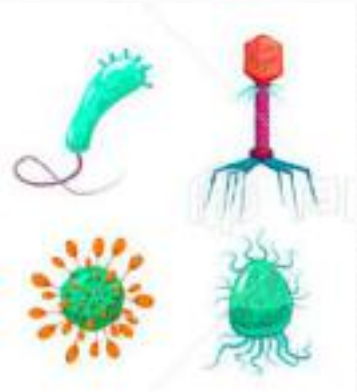


Principles of bacterial vaccines

- Bacterial diseases can be prevented by using immunizations
- that induce either active or passive immunity.
- Active immunity is induced by vaccines prepared from bacteria or
- their products.
- **Passive immunity** is provided by the administration of preformed antibody in preparations called immune globulins.
- The immune globulins useful against bacterial diseases.
- **Passive–active immunity** involves giving both immune globulins to provide immediate protection and a vaccine to provide long-term protection.

Active Immunity

- Bacterial vaccines are composed of capsular polysaccharides, inactivated protein exotoxins (toxoids), killed bacteria, or live, attenuated bacteria.

TABLE 12–1 Current Bacterial Vaccines

Usage	Bacterium	Disease	Antigen
Common usage	*Corynebacterium diphtheriae*	Diphtheria	Toxoid
	Clostridium tetani	Tetanus	Toxoid
	Bordetella pertussis	Pertussis (whooping cough)	Acellular (purified proteins) or killed organisms
	Haemophilus influenzae	Meningitis	Capsular polysaccharide conjugated to carrier protein
	Streptococcus pneumoniae	Pneumonia	Capsular polysaccharide or capsular polysaccharide conjugated to carrier protein
	Neisseria meningitidis	Meningitis	Capsular polysaccharide or capsular polysaccharide conjugated to a carrier protein
Special situations	*Salmonella typhi*	Typhoid fever	Live organisms or capsular polysaccharide
	Vibrio cholerae	Cholera	Killed organisms
	Yersinia pestis	Plague	Killed organisms
	Bacillus anthracis	Anthrax	Partially purified proteins
	Mycobacterium bovis (BCG)	Tuberculosis	Live organisms
	Francisella tularensis	Tularemia	Live organisms
	Rickettsia prowazekii	Typhus	Killed organisms
	Coxiella burnetii	Q fever	Killed organisms

TABLE 12–2 Vaccines Recommended for Children Age 0–6 Years[1]

Bacterial Vaccines	Viral Vaccines
Diphtheria toxoid, tetanus toxoid, acellular pertussis (DTaP)	Hepatitis A
Haemophilus influenzae type b (Hib)	Hepatitis B
Meningococcal	Influenza
Pneumococcal	Measles, mumps, rubella (MMR)
	Poliovirus, inactivated
	Rotavirus
	Varicella

[1]Vaccines are listed in alphabetical order. A complete description of the vaccine schedule is available on the Centers for Disease Control and Prevention Web site, www.cdc.gov.

Capsular Polysaccharide Vaccines

- 1) Both versions of the vaccine against **Streptococcus**
- **pneumoniae** contain the capsular polysaccharide of the bacteria as the immunogen.
- One version contains the capsular polysaccharide of the 23 most prevalent serotypes.
- It is recommended for persons older than 60 years of age and adult patients of any age with such chronic diseases as diabetes and cirrhosis or with compromised spleen function or splenectomy.
- A second version containing the capsular polysaccharide of 13 pneumococcal serotypes coupled to a carrier protein (diphtheria toxoid) is available for the protection of young children who do not respond well to the unconjugated vaccine.

- A potential problem regarding the use of the pneumococcal vaccine (or a vaccine against any organism with multiple serotypes) is that of serotype replacement.
- Will the vaccine reduce the incidence of disease caused by the serotypes in the vaccine but not the overall incidence of disease because other serotypes that are not in the vaccine will now cause
- disease?
- An increase in invasive pneumococcal disease caused by serotype 19A, a serotype not in the previous vaccine, was observed.
- In view of this, serotype 19A is included in the current 13 serotype vaccine

Neisseria meningitidis vaccine

- (2) Neisseria meningitidis vaccine contains capsular polysaccharide of four important types (A, C, W-135, and Y).
- Two forms of the vaccine are available: one contains the polysaccharides conjugated to a carrier protein (diphtheria toxoid), and the other contains only the polysaccharides.
- It is given when there is a high risk of meningitis (e.g., during an outbreak, when students enter college and are living in a dormitory, when military recruits enter boot camp, or for travelers to areas where meningitis is hyper endemic).

Haemophilus influenzae vaccine

- (3) Haemophilus influenzae vaccine contains the type b polysaccharide conjugated to diphtheria toxoid or other carrier protein.
- It is given to children between the ages of 2 and 15 months to prevent meningitis.
- The capsular polysaccharide alone is a poor immunogen in young children, but coupling it to a carrier protein greatly enhances its immunogenicity.
- A combined vaccine consisting of this vaccine plus the diphtheria, tetanus, and pertussis (DTP) vaccines is available.

Typhoid fever vaccine

- (4) One of the vaccines against typhoid fever contains the capsular polysaccharide of Salmonella typhi.
- It is indicated for persons living or traveling in areas where there is a high risk of typhoid fever and for persons in close contact with either infected patients or chronic carriers

Toxoid Vaccines

- (1) **Corynebacterium diphtheriae vaccine** contains the toxoid (formaldehyde-treated exotoxin).
- Immunization against diphtheria is indicated for every child and is given in three doses at 2, 4, and 6 months of age, with boosters given 1 year later and at intervals thereafter.
- (2) **Clostridium tetani vaccine** contains tetanus toxoid
- and is given to everyone both early in life and later as boosters for protection against tetanus.
- (3) **Bordetella pertussis vaccine** contains pertussis toxoid
- but includes other proteins as well.

Purified Protein Vaccines

- (1) There are two types of **B. pertussis vaccines**: an acellular vaccine containing purified proteins and a vaccine containing whole killed bacteria.
- The acellular vaccine is now recommended in the United States. The principal antigen in the acellular vaccine is inactivated pertussis toxin (pertussis toxoid), but other proteins, such as filamentous hemagglutinin and pertactin, are also required for full protection.
- Pertussis toxin for the vaccine is inactivated genetically by introducing two amino acid changes that eliminate its toxic (ADP-ribosylating) activity but retain its antigenicity.

- It is the first vaccine to contain a genetically inactivated toxoid.
- The vaccine is indicated for every child as a protection against whooping cough. It is usually given in combination with diphtheria and tetanus toxoids (DTP or DTaP vaccine).

(2) **Bacillus anthracis vaccine** contains "protective antigen" purified from the organism. It is given to persons whose occupations place them at risk of exposure to the organism

- **Borrelia burgdorferi „OspA":** Two monovalent recombinant vaccines based on outer surface protein A (OspA) serotype-1 derived from B. burgdorferi
-

Live, Attenuated Bacterial Vaccines

- (1) The vaccine against tuberculosis contains a live, attenuated strain of Mycobacterium bovis called BCG and, in some countries, is recommended for children at high risk for exposure to active tuberculosis.
- (2) One of the vaccines against typhoid fever contains live, attenuated S. typhi.
- It is indicated for persons living or traveling in areas where there is a high risk of typhoid fever and for persons in close contact with either infected patients or chronic carriers.
- (3) The vaccine against tularemia contains live, attenuated Francisella tularensis organisms and is used primarily in people who are exposed in their occupation, such as laboratory personnel, veterinarians, and hunters.
- Vibrio cholerae: (O1)

Killed Bacterial Vaccines

- (1) Vibrio cholerae vaccine contains killed organisms and is given to persons traveling to areas where cholera is endemic.
- (2) Yersinia pestis vaccine contains killed organisms and is indicated for persons at high risk for contracting plague.
- (3) The vaccine against typhus contains killed Rickettsia rickettsiae organisms and is used primarily to immunize members of the armed forces.
- The vaccine against Q fever contains killed Coxiella burnetii organisms and is used to immunize those who are at high risk for being exposed to animals infected with the organism.

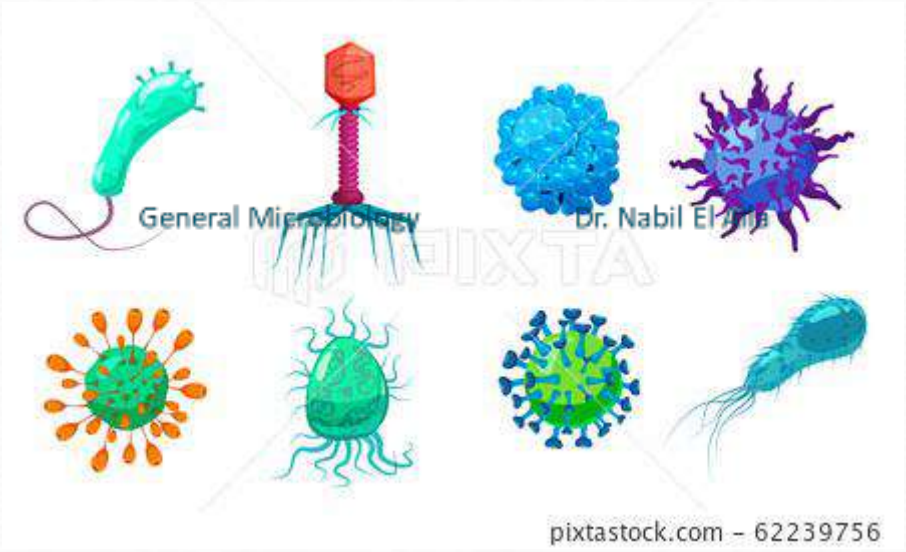
Passive Immunity

- **(1) Tetanus antitoxin** is used in the treatment of tetanus and in its prevention (prophylaxis). In treatment, because the goal is to neutralize any unbound toxin to prevent the disease from getting worse, the antitoxin should be given promptly.
- **(2) Botulinum antitoxin** is used in the treatment of botulism.

Because the antitoxin can neutralize unbound toxin to prevent the disease from progressing, it should be given promptly. It contains antibodies against botulinum toxins A, B, and E, the most commonly occurring types.

- (3) **Diphtheria antitoxin** is used in the treatment of diphtheria. The antitoxin can neutralize unbound toxin to prevent the disease from progressing; therefore, the antitoxin should be given promptly.
- Monoclonal antibody against exotoxin B of Clostridium difficile

End of Lecture



Bacterial Vaccines

Active Immunity

- **A. Capsular Polysaccharide Vaccines**

  - ***Streptococcus pneumoniae*** **(23) (13) (19A)**

  - ***Neisseria meningitidis*** **„A, C, W-135, Y“**

    (Group B, factor H binding protein)

  - ***Haemophilus influenzae***

  - ***Salmonella typhi*** **(type b)**

Active Immunity

- **B. Toxoid Vaccines**

  - Corynebacterium diphtheriae

  three doses at 2, 4, and 6 months of age

  - Clostridium tetani

  - Bordetella pertussis

Active Immunity

- **C. Purified protein vaccines**

- *B. pertussis* acellular vaccine

„genetically inactivated toxoid“

„DTP or DtaP“

- *Borrelia burgdorferi* „OspA“

„made by recombinant DNA technology“

- *Bacillus anthracis* vaccine

„ protective Ag“

Active Immunity

- **Live, Attenuated Bacterial Vaccines**

  - ***M. tuberculosis*: BCG**

  - ***S. typhi***

  - ***Francisella tularensis***

  - ***Vibrio cholerae*: (O1)**

Active Immunity

- **Killed Bacterial Vaccines**

  - ***Vibrio cholerae*** **(not in USA)**

  - ***Yersinia pestis***

  - ***Rickettsia rickettsiae or prowazekii***

    **** primarily for armed forces***

  - ***Coxiella burnetii***

Passive Immunity

- **Tetanus antitoxin:** **(passive–active immunity)**
- **Botulinum „A, B, E“** ***hypersensitivity??***
- **Diphtheria antitoxin** ***hypersensitivity??***
- **Monoclonal antibody against exotoxin B of *Clostridium difficile***

Sterilization & Disinfection

Sterilization, Disinfection & Standard precautions

- ***Standard precautions include:***
  - hand hygiene
  - respiratory hygiene and cough etiquette
  - safe injection practices
  - proper disposal of needles and scalpels

✓ Further, if exposure to body fluids or aerosols is likely, personal protective equipment (PPE) should be used

Principles of Sterilization & Disinfection

- **Sterilization:**
  - is the killing or removal of all microorganisms, including bacterial spores, which are highly resistant

- **Disinfection:**
  - is the killing of many, but not all, microorganisms
  - Disinfectants that on the surface of skin and mucous membranes are called **antiseptics**.

Clinical Use of Disinfection and Sterilization

Rate of killing:

$N \propto 1/CT$

Clinical Use	Commonly Used Disinfectant or Method of Sterilization
Disinfect surgeon's hands prior to surgery	Chlorhexidine
Disinfect surgical site prior to surgery	Iodophor
Disinfect skin prior to venipuncture or immunization	70% ethanol
Disinfect skin prior to blood culture or inserting vascular catheter	Tincture of iodine followed by 70% ethanol, or iodophor, or chlorhexidine
Cleanse wounds	Thimerosal, chlorhexidine, hydrogen peroxide
Cleanse burn wounds	Silver sulfadiazine
Cleanup of blood spill from a patient with hepatitis B or C (disinfect area)	Hypochlorite (bleach, Clorox)
Sterilize surgical instruments and heat-sensitive materials (e.g., endoscopes, respiratory therapy equipment)	Ethylene oxide or glutaraldehyde
Sterilize non–heat-sensitive materials (e.g., surgical gowns, drapes)	Autoclave
Sterilize intravenous solutions	Filtration
Disinfect air in operating room (when not in use)	Ultraviolet light
Disinfect floor of operating room	Benzalkonium chloride (Lysol)
Disinfect stethoscope	70% ethanol
Preservative in vaccines	Thimerosal

Chemicals

- **Germicides are grouped according to potency:**
  - Sterilants
  - High-level disinfectants (same as sterilants, yet used shorter times)
  - Intermediate level disinfectants
  - Low-level disinfectants

Table 5.3 Usual Pattern of Antimicrobial Activity of Germicides

	Bacteria			Fungi	Viruses	
Category of Germicide	Endospores	Mycobacteria	Others	All	Nonlipid	Lipid
Sterilant	yes	yes	yes	yes	yes	yes
High Level	some	yes	yes	yes	yes	yes
Intermediate Level	no	varies	yes	yes	varies	yes
Low Level	no	no	varies	varies	no	varies

Chemical Agents

- **The phenol coefficient**
- **Disruption of cell membrane**
  - Alcohol
  - Detergents
  - Phenols
- **Modification of proteins**
  - Chlorine - hydrogen peroxide
  - Iodine - Formaldehyde & Glutaraldehyde
  - Heavy metals - Ethylene oxide
  - Acids & Alkalis
- **Modification of Nucleic acids**
  - dyes

Chemical Agents

- **Disruption of cell membrane:**
  - ***Alcohol:*** requires the presence of water for maximal activity
  - ***Detergents:*** quaternary ammonium compounds (e.g., benzalkonium chloride) are cationic detergents widely used for skin antisepsis.
  - ***Phenols:*** phenols damage membranes & also denature proteins (e.g. chlorhexidine)

- **Modification of Nucleic acids:**
  - ***Dyes:*** crystal violet (antiseptic for skin fungal infections), & malachite green (LJ medium)

Chemical Agents

- **Modification of proteins**
  - ***Chlorine:*** is a powerful oxidizing agent (hypochlorite)
  - ***Hydrogen peroxide***: antiseptic working as an oxidizing agent that attacks sulfhydryl groups
  - ***Iodine:*** is an oxidant that inactivates sulfhydryl-containing enzymes (Tincture of iodine, Iodophors)
  - ***Formaldehyde & Glutaraldehyde:*** denatures proteins and nucleic acids (glutaraldehyde more effective and less toxic)
  - ***Heavy metals:*** act by binding to sulfhydryl groups (thimerosal and merbromin used as skin antiseptics. Silver nitrate drops)
  - ***Ethylene oxide:*** kills by alkylating both proteins and nucleic acids (mutagen & carcinogen)
  - ***Acids & Alkalis:*** kill by denaturing proteins (TB)

Physical Agents

- **Heat** „ Moist Heat”

Heat is still one of the most useful methods

- **Boiling:** for 10 min at 100°C destroys most (exception??)
- **Pasteurization:** significant # reduction
  - originally 62°C for 30 min
  - today HTST: 72°C for 15 sec (flash pasteurization)
- **UHT:** method technically not pasteurization (since it sterilizes): rapid heating to 140°C for several seconds, followed by rapid cooling

Moist Heat cont.

- ***Autoclave:*** Pressurized steam reaches higher temperatures, leading to sterilization.
  - Normal autoclave conditions: 121°C for 15 min.
  - Flash autoclaving: 135°C for 3 min.
  - Prion destruction: 132°C for 4.5 hours.

Dry Heat

- Takes much longer to kill

  - Flaming a wire inoculating loop

  - Glassware is heated in oven for 2-3 hours at 160°C to 170 °C

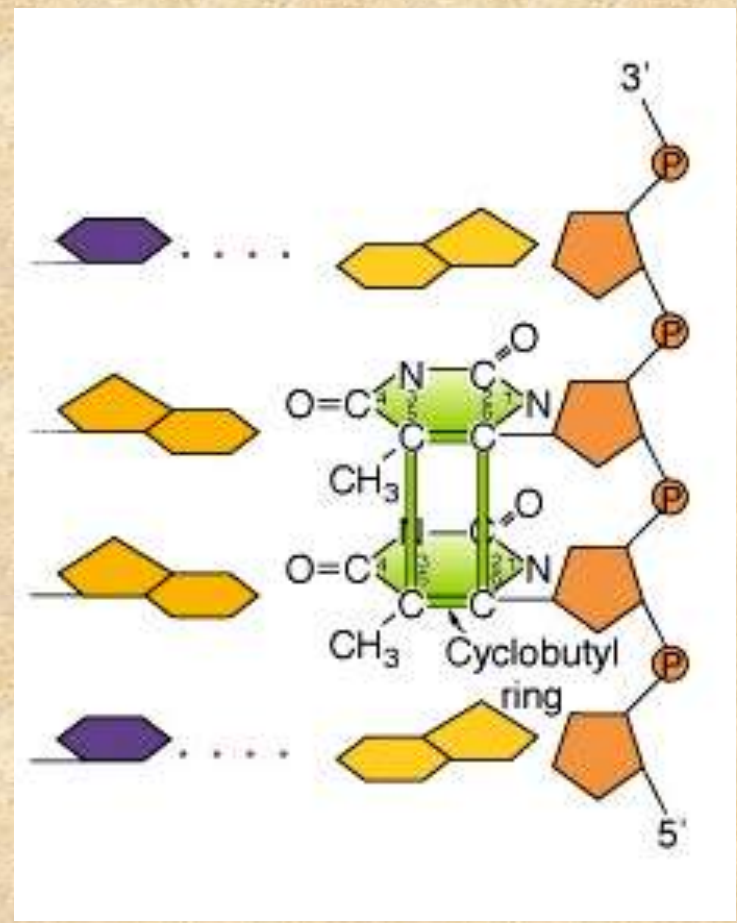
Radiation

- **UV Irradiation**

  - Thymine dimer formation

  - Actively dividing organisms are particularly sensitive because thymine dimers prevent proper replication

  - Use at close range to directly exposed microorganisms



Radiation

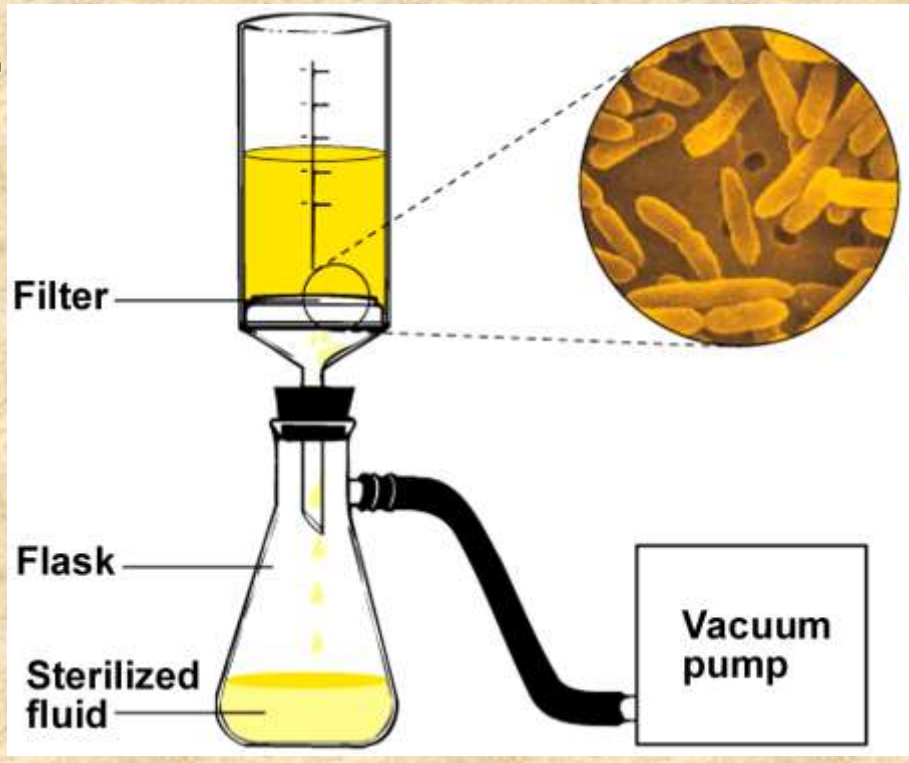
- **Gamma Irradiation**

- Ionizing radiation that causes reactive molecules and free radicals
- Commonly used source: Cobalt-60 radioisotope
- Very sensitive are *Salmonella* and *Pseudomonas*
- Also used as “cold pasteurization” of food.

Filtration

- **Filtration of fluids** with membrane filters (pore size for bacteria 0.2 – 0.4 μm)

- **Filtration of air** using high-efficiency particulate air (HEPA) filters *(effective to 0.3 μm)*
  - Laminar flow hood



Preservation of Perishable Products

- **Chemicals**:
  - Non-food items use germicides
  - Food items use weak acids (i.e. benzoic, sorbic, acetic,)
  - Nitrates are typically used to preserve meats
- **Low-temperature:**
  - Slows enzymatic reactions
  - Freezing forms ice crystals that damage microbial cells
- **Reducing water availability:**
  - Adding salt or sugar
  - Drying food (desiccation, lyophilization)